514 Rec'd PCT/PTO 14 MAR 2000
09/508658

Practitioner's Docket No. U 012653-9

CHAPTER II

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT/FI98/00749	23 SEPT. 1998	23 SEPT. 1997
INTERNATIONAL APPLICATION NO. CLAIMED	INTERNATIONAL FILING DATE	PRIORITY DATE

NOVEL GENE DEFECTIVE IN APECED AND ITS USE
TITLE OF INVENTION

Kai KROHN; Maarit HEINO; Part PETERSON; Hamish SCOTT; Stylianos ANTONARAKIS; Maria LALIOTI; Nobuyoshi SHIMIZU; Jan KUDOH
APPLICANT(S)

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

NOTE: The completion of those filing requirements that can be made at a time later than 30 months from the priority date results from the Commissioner exercising his judgment under the authority granted under 35 USC 371(d). The filing receipt will show the actual date of receipt of the last item completing the entry into the national phase. See 37 C.F.R. §1.491 which states: "An international application enters the national state when the applicant has filed the documents and fees required by 35 USC 371(c) within the periods set forth in § 1.494 and § 1.495."

CERTIFICATION UNDER 37 C.F.R. 1.10*
*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date MARCH 14, 2000, in an envelope as "Express Mail Post Office to Addressee," Mailing Label Number EL386267956US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

JENNIFER RASHKIN
(type or print name of person mailing paper)

Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** *Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

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WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. §1.10* ***must*** *be used (since international application papers are not covered by an ordinary certificate of mailing - See 37 C.F.R. §1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 USC 371 otherwise the submission will be considered as being made under 35 USC 111. 37 C.F.R. § 1.494(f).

1. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. 371:

   a. [x] This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).

   b. [x] The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

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2.Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
[ ]*	TOTAL CLAIMS	24 - 20 =	4	x $ 18.00 =	$72.00
	INDEPENDENT CLAIMS	4 - 3 =	1	x $ 78.00 =	78.00
	MULTIPLE DEPENDENT CLAIM(S) (if applicable) + $260.00				
BASIC FEE**	[ ] U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: [ ] and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(2) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 CFR 1.492(a)(4)) ..... $96.00 [ ] and the above requirements are not met (37 CFR 1.492(a)(1)) ..... $670.00 [X] U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: [ ] has been paid (37 CFR 1.492(a)(2)) ..... $690.00 [X] has not been paid (37 CFR 1.492(a)(3)) ..... $970.00 [ ] where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 CFR 1.492(a)(5)) $840.00			$970.00
	Total of above Calculations				=$1,120.00
SMALL ENTITY	Reduction by ½ for filing by small entity, if applicable. Affidavit must be filed. (note 37 CFR 1.9, 1.27, 1.28)				-
	Subtotal				
	Total National Fee				$
	Fee for recording the enclosed assignment document $40.00 (37 CFR 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				
TOTAL	Total Fees enclosed				$1,120.00

*See attached Preliminary Amendment Reducing the Number of Claims.

i. [X] A check in the amount of $1,120.00 to cover the above fees is enclosed.
ii. [ ] Please charge Account No. _____ in the amount of $ _____.
A duplicate copy of this sheet is enclosed.

**WARNING: *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b)*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. [X] A copy of the International application as filed (35 U.S.C. 371(c)(2)):

NOTE: *Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.*

a. [ ] is transmitted herewith.
b. [ ] is not required, as the application was filed with the United States Receiving Office.
c. [X] has been transmitted
i. [X] by the International Bureau.
Date of mailing of the application (from form PCT/IB/308): APRIL 1, 1999.
ii. [ ] by applicant on _____.
Date

4. [X] A translation of the International application into the English language (35 U.S.C. 371(c)(2)):
a. [X] is transmitted herewith.
b. [ ] is not required as the application was filed in English.
c. [ ] was previously transmitted by applicant on _____.
Date
d. [ ] will follow.

5. [X] Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

a. [ ] are transmitted herewith.
b. [ ] have been transmitted
   i. [ ] by the International Bureau.
   Date of mailing of the amendment (from form PCT/IB/308): _____.
   ii. [ ] by applicant on _____.
   Date
c. [X] have not been transmitted as
   i. [X] applicant chose not to make amendments under PCT Article 19.
   Date of mailing of Search Report (from form PCT/ISA/210): JAN. 25, 1999.
   ii. [ ] the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. [X] A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. 371(c)(3)):
a. [ ] is transmitted herewith.
b. [ ] is not required as the amendments were made in the English language.
c. [X] has not been transmitted for reasons indicated at point 5(c) above.

7. [X] A copy of the international examination report (PCT/IPEA/409)
[X] is transmitted herewith.
[ ] is not required as the application was filed with the United States Receiving Office.

8. [X] Annex(es) to the international preliminary examination report
a. [X] is/are transmitted herewith.
b. [ ] is/are not required as the application was filed with the United States Receiving Office.

9. [X] A translation of the annexes to the international preliminary examination report
a. [ ] is transmitted herewith.
b. [X] is not required as the annexes are in the English language.

10. [X] An oath or declaration of the inventor (35 U.S.C. 371(c)(4)) complying with 35 U.S.C. 115
    a. [ ] was previously submitted by applicant on _____. Date
    b. [ ] is submitted herewith, and such oath or declaration
        i. [ ] is attached to the application.
        ii. [ ] identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. 1.70.
    c. [X] will follow.

Other document(s) or information included:

11. [X] An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):
    a. [X] is transmitted herewith.
    b. [ ] has been transmitted by the International Bureau.
    Date of mailing (from form PCT/IB/308): _____.
    c. [ ] is not required, as the application was searched by the United States International Searching Authority.
    d. [ ] will be transmitted promptly upon request.
    e. [ ] has been submitted by applicant on _____. Date

12. [X] An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98:
    a. [X] is transmitted herewith.
    Also transmitted herewith is/are:
    [X] Form PTO-1449 (PTO/SB/08A and 08B).
    [X] Copies of citations listed.
    b. [ ] will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. 371(c).
    c. [ ] was previously submitted by applicant on _____. Date

13. [ ] An assignment document is transmitted herewith for recording.

A separate [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or [ ] FORM PTO 1595 is also attached.

_____
_____
_____

14. [X] Additional documents:
    a. [X] Copy of request (PCT/RO/101)
    b. [X] International Publication No. WO99/15559
        i. [ ] Specification, claims and drawing
        ii. [X] Front page only
    c. [X] Preliminary amendment (37 C.F.R. § 1.121)
    d. [X] Other

Form PCT/IB/306; Form PCT/IB/308; Form PCT/IB/332

15. [X] The above checked items are being transmitted
    a. [X] before 30 months from any claimed priority date.
    b. [ ] after 30 months.

16. [ ] Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on _____, namely:

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

NOTE: "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

[X] The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 12-0425 .

[X] 37 C.F.R. 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

[ ] 37 C.F.R. 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must

only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

[X] 37 C.F.R. 1.17 (application processing fees)
[X] 37 C.F.R. 1.17(a)(1)-(5)(extension fees pursuant to § 1.136(a).
[X] 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: *Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).*

NOTE: *37 C.F.R. 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.*

[X] 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

SIGNATURE OF PRACTITIONER

Reg. No.: 25,858

William R. Evans
(type or print name of practitioner)
c/o Ladas & Parry
26 West 61st Street
P.O. Address

Tel. No.: (212) 708-1930

Customer No.:

New York, NY 10023

03 Rec'd PCT/PTO 14 MAR 2000

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PCT/FI98/00749	23 SEPT. 1998	23 SEPT. 1997
INT'L APPLICATION NO.	INT'L FILING DATE	PRIORITY DATE CLAIMED

NOVEL GENE DEFECTIVE IN APECED AND ITS USE
TITLE OF INVENTION

Kai KROHN; Maarit HEINO; Part PETERSON; Hamish SCOTT; Stylianos ANTONARAKIS; Maria LALIOTI; Nobuyoshi SHIMIZU; Jan KUDOH
APPLICANT(S)

Attorney Docket: U 012653-9

Commissioner of Patents and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Please amend the above application as follows.

In the Claims

Claim 3, line 1, delete " or 2"

Claim 7, line 1, delete "or 6"

Claim 8, line 1, delete "any of claims 5 to 7" and substitute therefor -- claim 5--

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CERTIFICATION UNDER 37 C.F.R. 1.10*
*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date MARCH 14, 2000 in an envelope as "Express Mail Post Office to Addressee," Mailing Label Number EL386267956US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

JENNIFER RASHKIN
(type or print name of person mailing paper)

Signature of person mailing paper

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Claim 11, line 1, delete "or 10"

Claim 13, line 1, delete "any one of claims 9 to 12" and substitute therefore --claim 9--

Claim 14, line 1, delete "any one of claims 9 to 13" and substitute therefor --claim 9--

Claim 15, line 1, delete "any one of claims 9 to 14" and substitute therefor --claim 9--

Claim 18, line 1, delete "or 17"

Claim 19, lines 1-2, delete "any one of claims 1 to 4" and substitute therefor --claim 1--

Claim 20, line 1, delete "any one of claims 5 to 7" and substitute therefor --claim 5--

Claim 21, lines 1-2, delete "any one of claims 1 to 4" and substitute therefor --claim 1--

Claim 22, lines 1-2, delete "any one of claims 1 to 4" and substitute therefor --claim 1--

23. (amended) Reagents reacting with the DNA sequence characterized by comprising the sequence id. no. 1 or a functional fragment or variant thereof encoding a protein having the same functional activity, or an functionally equivalent isolated DNA sequence hybridizable thereto or the protein of claim 5 or with reagents therewith.

Respectfully submitted,

William R. Evans
c/o Ladas & Parry
26 West 61st Street
New York, NY 10023
Reg. No. 25,858 (212) 708-1930

Form B

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(c))—SMALL BUSINESS CONCERN

Docket Number (Optional)

Applicant or Patentee: Finnish Immunotechnology Ltd.
Serial or Patent No.:
Filed or Issued:
Title: Novel gene defective in apeced and its use

I hereby declare that I am

- [ ] the owner of the small business concern identified below:
- [X] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN Finnish Immunotechnology Ltd.
ADDRESS OF SMALL BUSINESS CONCERN Lenkkeilijänkatu 8, FIN-33530 Tampere, Finland

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

- [X] the specification filed herewith with title as listed above.
- [ ] the application identified above.
- [ ] the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights in the invention must file separate verified statements averring to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization having any rights in the invention is listed below:

- [ ] no such person, concern, or organization exists.
- [ ] each such person, concern or organization is listed below.

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Pekka Sillanaukee
TITLE OF PERSON IF OTHER THAN OWNER chief executive officer
ADDRESS OF PERSON SIGNING Lenkkeilijänkatu 8, 33520 TAMPERE FINLAND
SIGNATURE _____ DATE March 7th, 2000

NOVEL GENE DEFECTIVE IN APECED AND ITS USE

Field of the invention

The present invention relates to a novel gene, a novel protein encoded by said gene, a mutated form of the gene and to diagnostic and therapeutic uses of the gene or a mutated form thereof. More specifically, the present invention relates to a novel gene defective in autoimmune polyendocrinopathy syndrome type I (APS I), also called autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) (MIM No. 240,300).

Background

Autoimmune polyglandular syndrome type I (APS I), also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), is a rare recessively inherited disease (MIM No. 240,300) that is more prevalent among certain isolated populations, such as Finnish, Sardinian and Iranian Jewish populations. The incidence of the disease among the Finns and the Iranian Jews is estimated to be 1:25000 and 1:9000, respectively, whereas only few cases in other parts of the world are found each year.

APECED is one of the two major autoimmune polyendocrinopathy syndromes. The causing factor of APECED has not yet been identified. The syndrome is characterized by lack of tolerance to numerous self-antigens and can therefore be considered as a prototype of organ-specific autoimmune diseases. In APECED, the patient develops chronic mucocutaneous candidiasis soon after birth, and later several organ-specific autoimmune diseases, mainly hypoparathyreoidism, Addison's disease, chronic atrophic gastritis with or without pernicious anemia, and in puberty gonadal dysfunction occur [Ahonen P, Clin. Genet. 27 (1985) 535-542]. An accepted criterion for diagnosis of APECED is the presence of at least two of the three main symptoms, Addison's disease, hypoparathyroidism and candidiasis, in patients [Neufeld, M. *et al.*, Medicine 60 (1981) 355-362]. Immunologically, the major findings are the presence of high-titer serum autoantibodies against the effected organs, antibodies against *Candida albicans*, and low or lacking T-cell responses toward candidal antigens [Blizzard, R. M. and Kyle M., J. Clin. Invest. 42 (1963) 1653-1660; Arulanantham, K. *et al.*, New Eng. J. Med. 300 (1979) 164-168; Krohn, K. *et al.*, Lancet 339 (1992) 770-773; Uibo R. *et al.*, J. Clin. Endocrinol. Metab. 78 (1994) 323-328]. The disease usually occurs in

childhood, but new tissue specific symptoms may appear throughout life [Ahonen, P. *et al.*, New Engl. J. Med. 322 (1990) 1829-1836]. APECED is not associated with a particular HLA haplotype, and both males and females are equally affected consistant with the autosomal recessive mode of inheritance.

The locus for the APECED gene has been mapped to chromosome 21q22.3 between gene markers D21S49 and D21S171 based on linkage analysis of Finnish families [Aaltonen, J. *et al.*, Nature Genet. 8 (1994) 83-87]. Recently, Börses *et al.* reported a maximum LOD score of 10.23 with marker D21S1912 just proximal to the gene PFKL, and thus by linkage disequilibrium studies the critical region for APECED can be considered to be less than 500 kb between markers D21S1912 and D21S171. Locus heterogeneity was not revealed by linkage analysis of non-Finnish families [Björses, P. *et al.*, Am. J. Hum. Genet. 59 (1996) 879-886].

For the APECED gene, the name "autoimmune regulator" or "AIRE" has been adopted by the scientific community after the priority date of the present application. Similarly the protein encoded by the AIRE gene is now called the "AIRE protein".

Physical maps of human chromosome 21q22.3 have been developed using YACs, and bacterial based large insert cloning vectors [Chumakov *et al.*, Nature 359 (1992) 380; Stone et al., Genome Res. 6 (1996) 218], and many laboratories have contributed to the construction of a transcription map of the whole chromosome and 21q22.3 in particular [Chen *et al.*, Genome Res. 6 (1996) 747-760; Yaspo *et al.*, Hum. Mol. Genet. 4 (1995) 1291-1304]. Numerous trapped exons from chromosome 21 specific cosmids and also physical contigs from the APECED critical region have been identified and partially characterized. In addition, a number of ESTs from the international human genome project have been mapped to the APECED critical region.

Recently, as part of the international efforts of generating the entire sequence of human chromosome 21 and international agreements on the immediate availability of this type of sequence data, the partial sequence of the APECED gene critical region was made available in GenBank by the Stanford Human Genome Center which is currently carrying out the sequencing of 1.0 Mb around the critical region of the APECED gene.

However, the precise location and the sequence of the APECED gene and the nature of the gene product have not so far been clarified. Thus

at present the diagnosis of APECED is based mainly on developed clinical symptoms and typical clinical findings, e.g. the presence of autoantibodies against adrenal cortex or steroidogenic enzymes P450c17 and/or P450scc. The linkage analysis is seldom used. Further, means for natal or presymptomatic diagnosis of the disease are not easily available, since the linkage analysis provides only an indirect data through known gene markers and requires samples from several family members in several generations. Additionally, the linkage analysis is tedious and can be performed only in specialized laboratories by highly-skilled personnel.

Also the mapping of the carriers of the disease gene is presently based on the linkage analysis and thus not readily available.

Summary of the invention

We have now identified a novel gene encoding a novel zinc finger protein, designated as autoimmune regulator 1 or AIR-1, which is mutated in APECED. The novel gene and protein allow further development of the diagnosis and therapy of diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED.

The object of the invention is to provide means which are useful in a diagnostic method and a gene therapeutic method in the diagnosis and treatment of diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED.

Another object of the invention is to provide a novel method for the diagnosis of diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED, including the pre- and postnatal diagnosis and the mapping of the carriers, the method being easy and reliable to perform.

The present invention relates to an isolated DNA sequence comprising the sequence id. no. 1 or a functional fragment or variant thereof, or a functionally equivalent isolated DNA sequence hybridizable thereto, the DNA sequence being associated with diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED. Preferably said isolated DNA sequence includes a gene defect responsible for APECED.

The present invention also relates to a protein comprising the amino acid sequence id. no. 2 or a functionally equivalent fragment or variant thereof, the protein being associated with diseases related to immune maturation and

regulation of immune response towards self and nonself, such as APECED. Said protein has distinct structural motifs, including the PHD finger motif (PHD), the LXXLL motif (L), proline-rich region (PRR), and cystein-rich region (CRR).

The present invention further relates to a method for the diagnosis of diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED, comprising detecting in a biological specimen the presence of a DNA sequence comprising the sequence id. no. 1 or a functional fragment or variant thereof, or a functionally equivalent DNA-sequence hybridizable thereto, the DNA sequence being associated with diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED.

The present invention further relates to the use of the above-identified DNA-sequences in the diagnosis of diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED.

The present invention further relates to a method for the diagnosis of diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED, comprising detecting in a biological specimen the presence or the absence of a protein comprising the sequence id. no. 2 or a functionally equivalent fragment thereof, the protein being associated with diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED.

The present invention further relates to the use of the above-identified protein or a functionally equivalent fragment thereof in the diagnosis of diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED.

The present invention further relates to the use of the above-identified DNA sequences in gene therapy or for the preparation of a pharmaceutical preparation useful in a gene therapy method of diseases related to immune maturation and regulation of immune response towards self and nonself, such as APECED.

Brief description of the drawings

Figure 1 shows a physical map of the APECED gene locus in the chromosome 21q22.3. Cosmids D1G8, D40G11, D9G11, D28B11, and D4G11, overlapping clones used for the genomic sequencing [Kudoh, J. *et al.*,

DNA Res. 4 (1997) 45 -52] are indicated by horizontal lines. The APECED gene located just proximal to the 5' end of the neighboring gene PFKL is indicated by a solid arrow. N indicates *Not*I sites. DNA marker D21S1912 is shown as open box.

Figure 2 shows the structures of the APECED gene and AIR proteins. (A) Cloning strategy of APECED cDNAs and the order of the exons in the APECED gene. DNA fragments amplified by PCR and 3'- and 5'-RACE are indicated by the lines. Exon 1' is the 5'-noncoding exon of the AIR-2 and AIR-3. An additional alternative splicing of AIR-3 in exon 10, resulting in an amino acid change in its downstream, is indicated by vertical lines. Each exon, except exon 1', is bordered by the common splice site consensus sequence, ag:gt. Mutations in the exon 2 and exon 6 are indicated by the arrows. (B) Schematic presentation of the three AIR proteins showing distinct structural motifs, including the PHD finger motif (PHD), the LXXLL motif (L), proline-rich region (PRR), and cystein-rich region (CRR).

Figure 3 shows electropherograms showing the sequence surrounding the mutations in the APECED gene. (A) Mutation analysis of a Swiss APECED family. The parents are heterozygous for the allele (normal "C" and abnormal "T"). The affected boy and girl show the "C" to "T" transition resulting in the "Arg" to "Stop" nonsense mutation at amino acid position 257. (B) Mutation analysis of two Finnish APECED patients. The patient MP is homozygous for the mutant allele (left), NP is heterozygous for the allele (right). (C) The patient NP shows the "A" to "G" transversion resulting in the "Lys" to "Glu" missense mutation at amino acid position 42. FLEB is a normal control.

Figure 4 shows the result of a restriction enzyme *Taq*I digestion assay demonstrating the R257stop mutation. Four APECED patients [HP1 (lane 1), HP2 (lane 2), NP (lane 6), and MP (lane 8)], the mothers of two families [HM (lane 5) and NM (lane 7)], two healthy siblings [HN1 (lane 3) and HN2 (lane 4)] of family H and normal controls [C1, C2 and C3 (lanes 9-11)] are shown. The APECED patients HP1, HP2 and MP are homozygotes for the R257stop mutation. The APECED patient NP is heterozygous for the R257stop mutation but is carrying a mutation at a different position in another allele of the APECED gene (shown above in Fig. 3C). Both mothers (HM and NM) and two healthy siblings (HN1 and HN2) are heterozygous for the R257stop mutation and therefore carriers of APECED but are not having the

disease. Two controls (C1 and C2) are both homozygous for normal alleles. Normal alleles produce a lower 225 bp fragment, the mutated fragment is upper band at 285 bp.

Figure 5 shows an amino acid sequence alignment for the PHD finger motif of AIR-1, Mi-2, and TIF1. The consensus amino acid residues conserved in the PHD finger motif is indicated by the bold letters underneath. The residues that are identical with AIR-1 (aa 299-340) are shown by the dots. GenBank accession nos. of Mi-2 and TIF1 are X86691 and AF009353, respectively.

Figure 6 is a Western blot showing the expression of AIR-1 in fetal liver. A sample of fetal liver was run on PAGE, transferred to a nitrocellulose filter and probed with sera as follows: Lane 1, control mouse serum, lane 2, control mouse serum absorbed with peptide AIR-1/2 (sequence id. no. 25), lanes 3 and 4, serum from a mouse immunized with peptide AIR-1/2 for four and six weeks, respectively and absorbed with peptide AIR-1/2, lanes 5 and 6, unabsorbed serum from a mouse immunized with peptide AIR-1/2 for four and six weeks, respectively. The strong band seen in lanes 5 and 6 represent the AIR-1 protein with a molecular weight of approx. 58 kD, the lower band is an approx. 20 kD breakdown product of the AIR protein. The bands seen in all lanes are non-specific.

Figure 7 shows the expression of the APECED mRNA (7A) or the AIR protein (7B, 7C and 7D) demonstrated by in situ hybridization (7A) or by immunohistochemistry (7B, 7C and 7D). Figure 7A shows APECED mRNA positive cells scattered in the medullary region of human thymus. Figure 7B shows similar cells with the same localization now stained for the AIR protein. Figure 7C is a higher magnification of 7B, showing the localization of the AIR protein in the nuclei. Note the speckled localiation pattern in the nuclei. Figure 7D shows the cytoplasmic localization of the AIR protein in a few cells in lymph node medulla.

Figure 8 shows the phenotypic characterization of the APECED reactive cells in thymus by double-immunofluorescence. The AIR protein is seen as red colour in the nuclei, forming typical speckled pattern with nuclear dots. In Figure 8A, the co-staining is with an antibody recognizing low molecular weight markers (AE1). The APECED positive cells fall into two types, one is expressing cytokeratin and is thus epithelial cell, the other one is non-epithelial and do not co-express cytokeratins. In Figure 8B an APECED

positive cell co-epresses a marker (CD83) typical for cells belonging to monocyte-macrophage-dendritic cell lineage.

Figure 9 shows the expression of the AIR protein, demonstrated by immunofluorescence, in mature, activated dendritic cells from peripheral blood. The expression of the AIR protein shows as distinct dots in the nuclei of dendritic cells.

Detailed description of the invention

The present invention is based on studies aiming for the identification and characterization of the gene defect in APECED. In the sequence studies, a cosmid/BAC (bacterial artificial chromosome) contig of 520 kb covering four gene markers D21S1460-D21S1912-PFKL-D21S154 [Kudoh, J. *et al.,* DNA Res. 4 (1997) 45-52] was constructed, and genomic sequencing in this region was performed [Kawasaki, K. *et al.*, Genome Res. 7 (1997) 250-261]. From this genomic sequence information the distance between D21S1912 and PFKL was determined to be approximately 140 kb (Fig. 1).

Using a computer program, such as GRAIL and GENSCAN [Uberbacher, E. C. and Mural, R. J., Proc. Natl. Acad. Sci. USA 88 (1991) 11261-11265; Burge, C. and Karlin, S., J. Mol. Biol. 268 (1997) 78-94], gene screening in the partial sequencing data within this region was performed. GENSCAN predicted several genes between D21S1912 and PFKL. One of these genes located just proximal to the PFKL gene contained the previously trapped exon HC21EXc33 [Kudoh, J. *et al.,* DNA Res. 4 (1997) 45-52] or MDC04M06 [Chen, H. *et al.*, Genome Res. 6 (1996) 747-760]. A set of primers for polymerase chain reaction (PCR) was then designed from the predicted exons. The PCR screening of various cDNA libraries using these primers allowed the isolation of a cDNA clone containing the exon HC21EXc33 (exon 13) from the thymus cDNA library (Fig. 2A).

A 3'-rapid amplification of cDNA ends (3'-RACE) and 5'-RACE using Marathon™ cDNA Amplification Kit (Clontech Laboratories Inc, California, USA) according to manufacturer's protocol from the thymus cDNA library was performed using a primer c33F (sequence id. no. 7) and a primer 1R (sequence id. no. 8), respectively.

Sequencing analysis revealed a unique sequence of 2027 bp in overlapping PCR products that contains a 1635-bp open reading frame (ORF) from methionine at nt 128 to a TAG stop codon at nt 1763 encoding a predic-

ted novel protein designated AIR-1, for autoimmune regulator 1. AIR-1 encodes a protein of 545 amino acids with a predicted isoelectric point of 7.32 and a calculated molecular mass of 57,723 (Fig. 2B).

A 5'-RACE from the thymus cDNA using a primer 4R (sequence id. no. 9) resulted in an alternatively spliced product. Furthermore, two types of the cDNA clones were amplified with a primer pair 3F/c33R (sequence id. no. 10/sequence id. no. 11) and these clones encode for AIR-2 and AIR-3 proteins, sequence id. no. 4 and sequence id. no. 6, respectively (Fig. 2A) (sequence id. no. 3 and sequence id. no. 5). The AIR-2 and AIR-3 proteins consist of 348 and 254 amino acids, respectively (Fig. 2B). These results suggest that the APECED gene is transcribed as at least three types of mRNA by alternative splicing and/or use of an alternative 5' exon within the gene. RT-PCR analysis [Griffin, H. G. and Griffin, A. M., PCR Technology. Current Innovations, CRC Press, 1994] revealed that the AIR-1 transcript is also expressed in fetal liver (data not shown).

The APECED gene is approximately 13-kb in length and contains 15 exons, including the exon 1' specific to AIR-2 and AIR-3. It is transcribed in the direction of centromere to telomere (Figs 1, 2A). Based on this information, PCR primers were designed to amplify each exon from the genomic DNA and a mutation analysis of Swiss and Finnish APECED families was performed. Sequence comparison identified two mutations in the APECED gene of the patients (Fig. 3). The first mutation changes an Arg codon (CGA) to a stop codon (TGA) at amino acid position 257 in exon 6. This mutation was designated as R257stop mutation. The second mutation is a missense mutation that derived from the maternal chromosome in one Finnish patient (NP): a Lys codon (AAG) changes to a Glu codon (GAG) at amino acid position 42 in exon 2. This mutation is designated as K42E mutation (Figs 2A, 3C).

The R257stop mutation destroys a *Taq*I restriction enzyme site and the K42E mutation introduces a novel *Taq*I site. Thus these two mutations can be easily demonstrated in one or both alleles by *Taq*I digestion or by digestion using another enzyme cleaving at the recognition site 5'-TCGA-3'(Fig. 4).

The AIR-1 protein has strong homology in certain domains to the major autoantigens (Mi-2) associated with the autoimmune disease dermatomyositis [Seeig, H. P. *et al.*, Arthritis Rheum. 38 (1995) 1389-1399; Ge, Q. *et al.*, J. Clin. Invest. 96 (1995) 1730-1737], Sp140, a protein from the nuclear body, an organelle involved in the pathogenesis of certain types of

leukemia, and which is also the target of antibodies in the serum of patients with the autoimmune disease primary bilary cirrhosis [Bloch, D. B. *et al.*, J. Biol. Chem. 271 (1996) 29198-29204]. In addition, the homologies extend to other nuclear proteins such as TIF1 [Le Douarin, B. *et al.*, EMBO J. 14 (1995) 2020-2033], LYSP100 [Dent, A. L. *et al.*, Blood 88 (1996) 1423-1426], and putative yeast and *C. elegans* proteins. The AIR-1 protein homologies are principally in two PHD finger motifs (amino acid 299 to 340 and 434 to 475) (Fig. 5). AIR-1 also contains a proline-rich region (amino acid 350 to 430) (Fig. 2B). The PHD finger is a cysteine-rich structure that is distinguished from the RING finger (C3HC4) and LIM domain (C2HC5) because it contains a consensus of C4HC3. [Aasland, R. *et al.*, Trends Biochem. Sci. 20 (1995) 56-59]. The PHD finger motif is found in a number of chromatin-associated proteins such as HRX that is involved in the t(11:17) translocation in acute leukemia [Chaplin, T. *et al.*, Blood 86 (1995) 2073-2076]. The proline-rich region is assumed to be involved in protein-protein interaction or DNA binding. The presence of the PHD finger and proline-rich regions indicates a function for AIRs as transcription regulatory proteins. However, the AIR proteins have no apparent nuclear translocation signal, and thus other proteins containing such signal may interact with AIR to translocate it to the nucleus. In fact, the AIR proteins also have the LXXLL motif that is a signature sequence to bind to nuclear receptors [Heery, D. M. *et al.*, Nature 387 (1997) 733-736] (Fig. 2B).

The clinical picture of APECED and the observed immunological abnormality with strong autoimmune response towards several target organs and antigens suggest that the product of the APECED gene has a central role in immune (ontogeny) maturation and regulation of immune response towards self and nonself.

According to the diagnostic method of the invention, the presence of the defective APECED gene can be detected from a biological sample by any known detection method suitable for detecting mutations. Such methods include the method described by Saiki *et al.* [Proc. Natl. Acad. Sci USA 86 (1989) 6230-6234) utilizing hybridization to an allele specific oligonucleotide probe, or modifications thereof; the method described by Newton, C. R. *et al.* [Nucl. Acids Res. 17 (1989) 2503-2516] using the DNA sequences or DNA-fragments of the invention as probes; the solid phase minisequencing method described by Syvänen *et al.* [Genomics 8 (1990) 684-692] in which use is made of a biotinylated probe; or the oligonucleotide ligation method described

by Landegren, U. *et al.* [Science 241 (1988) 1077-1080]. Methods include the denaturing gradient gel electrophoresis (DGGE) [Fischer, S.G. and Lerman, L.S., PNAS 80 (1983) 1579-1583] or a modification of this method, constant denaturant gel electrophoresis (CDGE) [Hoving *et al.*, Genes Chromosomes Cancer 5 (1992) 97-103]. The mutation separation principle of DGGE and CDGE is based on the melting behavior of the DNA double helix of a given fragment.

Since the mutations of the APECED gene involve a site sensitive to *Taq*I digestion, the mutation are preferably detected in one or both alleles by *Taq*I digestion or by digestion using another enzyme cleaving at recognition site 5'-TCGA-3' The chemical mismatch cleavage for mutation analysis can be used [Grompe, M. *et al.*, Proc. Natl. Acad. Sci. USA 86(15)(1989) 5888-5892].

In the diagnostic method of the invention the biological sample can be any tissue or body fluid containing cells, such as blood, e.g. umbilical cord blood, separated blood cells, such as lymphocytes, B-cells, T-cells etc., biopsy material, such as fetal liver or thymus biopsy, sperm, saliva, etc. The biological sample can be, where necessary, pretreated in a suitable manner known to those skilled in the art.

When the DNA sequence of the present invention is used therapeutically any techniques presently available for gene therapy can be employed. Accordingly, in the technique known as *ex vivo* therapy patient cells (e.g. umbilical cord blood from the fetus) with the defective gene are taken from the patient, DNA sequences encoding the normal (healthy) gene product incorporated in a carrier vector are transducted or transfected to the cells and the cells are returned to the patient. If the techniques known as *in situ* therapy is used, the DNA sequences encoding the normal gene product are first inserted to a suitable carrier vector, and the carrier is then introduced to the affected tissue, such as peripheral blood, liver or bone marrow. The carrier vector used can be a retrovirus vector, an adeno virus vector, an adeno associated virus (AAV) vector or an eucaryotic vector. The therapy can be performed intra utero or during adult life. Depending on the cells to be treated these techniques lead either to a transient cure, where cells from affected organ are treated, or to a permanent cure, in case of the treatment of stem cells.

The present invention provides means for an easy and more rapid diagnosis of the diseases related to immune maturation and regulation of

immune response towards self and nonself, such as APECED, and, specifically, enables prenatal diagnosis and carrier diagnosis. Furthermore, it provides a background for therapy.

The invention is now elucidated by the following non-limiting examples.

Example 1

Localization of the APECED gene

Genomic sequencing of cosmid DNAs was performed by the shotgun method described by Kawasaki, K. *et al.*, Genome Res. 7 (1997) 250-261. Cosmids D1G8, D40G11, D9G11, D28B11, and D4G11 and gene marker D21S1912 are described by Kudoh, J. *et al.*, DNA Res. 4 (1997) 45-52].

cDNA cloning

The phage DNAs prepared from human thymus cDNA library (Clontech, HL1127a) were used as a PCR template. 20 ng of phage DNA which represents approximately $4x10^8$ phages was added to a 10 ml of reaction mixture containing 1x buffer [16mM $(NH_4)_2SO_4$, 50mM Tris-HCl, pH 9.2, 1.75 mM $MgCl_2$, 0.001% (w/v) gelatin), 0.2 mM each of dNTPs, 1M Betaine (Sigma), 0.35 U of Tap and Pwo DNA polymerase (EXpand Long Template PCR System, Boehringer Mannheim), and 0.5 mM of each of the primers, 2F and c33R, 2F and 4R, and 2F' and 2R', respectively.

The cDNA fragment was amplified by PCR using the following conditions: 94°C for 3 min., 35 cycles of 94°C for 30 sec, 60 °C for 30 sec in 2F/c33R and 2F/4R or 65°C for 30 sec in 2F'/2R', and 68°C for 90 sec. 3'- and 5'-RACE were carried out by Marathon cDNA Amplification Kit (Human Thymus; Clontech). PCR reaction was performed in a 10 µl volume containing 1x buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin), 0.2 mM each of dNTPs, 0.25 U of AmpliTaq Gold polymerase (Perkin-Elmer), and 0.5 mM of each of the exon-specific primers. 3'-RACE product was amplified by PCR with the following conditions: 95°C for 9 min., 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec.

The cDNA fragments were sequenced by the dye deoxy terminator cycle sequencing method (according to ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit protocol P/N 402078, Perkin Elmer Corporation, California) using specific primers, 2F and c33R, and AmpliTaq/FS

DNA polymerase (Perkin-Elmer), and then analyzed by using an automatic DNA sequencer (Applied Biosystems 377). Primer sequences used were

1R: 5'-GTTCCCGAGTGGAAGGCGCTGC-3' (sequence id. no. 8)

2F: 5'-GGATTCAGACCATGTCAGCTTCA-3' (sequence id. no. 12)

3F: 5'-GAGTTCAGGTACCCAGAGATGCTG-3' (sequence id. no. 10)

c33R: 5'-CTCGCTCAGAAGGGACTCCA-3' (sequence id. no. 11)

4R: 5'-AGGGGACAGGCAGGCCAGGT-3' (sequence id. no. 9)

2F': 5'-GTGCTGTTCAAGGACTACAAC-3' (sequence id. no. 13)

2R': 5'-TGGATGAGGATCCCCTCCACG-3' (sequence id. no. 14)

AP1: 5'-CCATCCTAATACGACTCACTATAGGGC-3' (sequence id. no. 15) and

c33F: 5'-GATGACACTGCCAGTCACGA-3' (sequence id. no. 7).

Example 2

Mutation analysis of the APECED gene

For the mutation analysis the DNA samples were purified from periferal blood mononuclear cells from patients with APECED and from suspected carriers of APECED and from normal healthy controls (according to Sambrook *et al.* 1989, Molecular Cloning. A Laboratory Manual. CSH Press) and subjected to PCR using primers specific for all identified exons.

For sequencing the mutated exons, PCR fragments, 6F/6R in exon 6 and 49300F/49622R in exon 2, were amplified by PCR with the following conditions: 95°C for 9 min., 35 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec, and 94°C for 3 min., 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 68°C for 30 sec, respectively. The PCR products were sequenced using specific primers

6F: 5'-TGCAGGCTGTGGGAACTCCA-3' (sequence id. no. 16)

6R: 5'-AGAAAAAGAGCTGTACCCTGTG-3' (sequence id. no. 17)

3R: 5'-TGCAAGGAAGAGGGGCGTCAGC-3' (sequence id. no. 18)

49300F: 5'-TCCACCACAAGCCGAGGAGAT-3' (sequence id. no. 19) and 49622R: 5'-ACGGGCTCCTCAAACACCACT-3' (sequence id. no. 20).

In the mutation analysis by sequencing, two Swiss and three Finnish (HP1, HP2 and MP) patients with APECED were homozygous for R257stop allele, whereas one Finnish patient (NP) was heterozygous for this mutation (Fig. 3A, B). The R257stop mutation of NP was derived from the

paternal chromosome. The second mutation, K42E mutation, was found in one Finnish patient (NP): a Lys codon (AAG) changes to a Glu codon (GAG) at amino acid position 42 in exon 2.(Figs 2A, 3C). This mutation derived from the maternal chromosome.

Example 3

Restriction enzyme TaqI analysis of two mutations in exons 2 and 6 of APECED gene

Analysis of the mutation sites in exons 2 and 6 in large series of individuals was performed using the restriction enzyme TaqI.The TaqI digestion for exons 2 and 6 was done as follows. Ten microlitres of amplification product was incubated at 65°C for 1 hour in 20 µl of reaction mixture containing 1x TaqI digestion buffer (New England Biolabs, NY, 100 µl/ml of BSA and 10U of TaqI enzyme (New England Biolabs, NY). After the digestion fragments were separated in 1.5% agarose gel and visualized by EtBr staining.

For exon 2, the fragment containing the mutation site K42E was amplified with primers GR1/2F and GR1/2R with the following conditions: 95°C for 3 min., 35 cycles of 94°C for 30 sec, 62°C for 30 sec and 72°C for 1 min. The 1x reaction mix used contained 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin), 0.2 mM each of dNTPs, 0.25 U of Dynazyme (Finnzymes, Finland), and 0.5 mM of each of the exon-specific primers. The normal allele produces a 312 bp fragment whereas the mutated allele gives a 133 bp and a 179 bp fragment. Primer sequences for GR1/2F and GR1/2R are 5'-TGGAGATGGGCAGGCCGCAGGGTG (sequence id. no. 21) and 5'-CAGTCCAGCTGGGCTGAGCAGGTG (sequence id. no. 22), respectively.

For exon 6, the fragment containing the R257stop mutation site was amplified with primers GR1/5IF and GR1/5IR with the same conditions described for exon 2 (see above). The normal allele produces a 225 bp fragment whereas the mutated allele gives a 285 bp fragment. Primer sequences for GR1/5IF and GR1/5IR are 5'-GCGGCTCCAAGAAGTGCATCCAGG (sequence id. no. 23) and 5'-CTCCACCCTGCAAGGAAGAGGGGC (sequence id. no. 24), respectively.

The screening of 50 Finnish and 50 Swiss healthy individuals did not reveal R257stop or K42E mutations by *Taq*I digestion. Similarly, PCR ana-

lysis of 20 unaffected Japanese was performed and no mutations were found in any of these positions. These results demonstrate that the APECED gene is responsible for the pathogenesis of APECED.

Mutations were found in the AIR-1 transcript but not in the AIR-2 and AIR-3 transcripts from all the APECED patients tested. Two Swiss and three Finnish (HP1, HP2 and MP) patients who are homozygous for the R257stop mutation completely lack functional AIR-1 protein but still have intact AIR-2 and AIR-3 proteins.

One common mutation seems responsible for the genetic defect in approximately 90% of the Finnish APECED cases and a haplotype analysis with the markers D21S141, D21S1912 and PFKL shows that the R257stop mutation is likely to be this common mutation [Björses, P. *et al.*, Am. J. Hum. Genet. 59 (1996) 879-886].

Example 4

Analysis of the AIR protein expression

In this example, synthetic peptides representing amino-acid sequences of the AIR-1 protein, were used to generate a polyvalent mouse antiserum against the AIR-1 protein.

For the peptide synthesis, two peptides were chosen according to the antigenicity prediction by Pepsort program (GCC package, Wisconsin, USA). The peptides AIR-1/2 and AIR-1/6 (TLHLKEKEGCPQAFH, sequence id. no. 25 and GKNKARSSSGPKPLV, sequence id. no. 26, respectively) representing exons 2 and 6, respectively, of the APECED gene were synthesized onto a branched lysine core (Fmoc8-Lys4-Lys2-Lys-betaAla-Wang resin, Calbiochem-Novabiochem, La Jolla, Ca, USA) resulting in an octameric multible antigen peptide (MAP) [Tam, J. P. *et al.*, Proc. Natl. Acad. Sci. USA 85 (1988) 5409-5413; Adermann, K. *et al.*, in Solid Phase Synthesis, Biological and Biomedical Applications, pp. 429-432, Ed. R. Epton, Mayflower Worldwide Ltd., Birmingham, 1994], Syntheses were performed by Fmoc (N-(9-fluorenyl)methoxycarbonyl) chemistry on a simultaneous multiple peptide synthesizer (SMPS 350, Zinsser Analytic, Frankfurt, Germany). Purity of MAPs was analyzed by reverse-phase HPLC (System Gold, Beckman Instruments Inc, Fullerton, CA, USA).

To obtain murine polyclonal antibodies, eight-week old Balb/c mice were immunized with an intraperitoneal injection of 25 micrograms of each

peptide in 0,4 ml of a 1:1 mixture of Freund's Complete Adjuvant (Difco Laboratories, Detroit, MI, USA) and physiological saline (NaCl, 0,15 M). One month later the animals were boosted with an intramuscular injection of 35 micrograms of antigens in Freund's incomplete adjuvant and saline (1:1) (0,2 ml were distributed into four sites). Three weeks later the peptides in a dose of 50 micrograms/mouse were administered intravenously and sera were obtained 7 days later.

For the production of EBV transformed B-cells, peripheral blood leukocytes were obtained from healthy control persons. The B-cells were transformed with EBV (Epstein-Barr virus) using standard protocol, and the cell lines were maintained in RPMI 1640, supplemented with 10% FCS (fetal calf serum). An aliquot of cells were stimulated for 12 hours with 10 mg/ml of phytohemagglutinin (PHA) to obtain mitogen-activated T-cells.

Tissue samples were obtained from stillborn fetuses at six months gestational age. Fetal liver, spleen, thymus and lymphnodes were homogenized, the homogenates were cleared with centrifugations (20 000 rpm for 20 minutes) and the samples were used for western blot analysis.

For analysis of polyclonal sera, Elisa and western blot analysis were performed. Microtitre ELISA plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with the peptides (1 micrograms /well in PBS, pH 7,5) at $4^{o}C$ overnight and blocked with 2 % of BSA in PBS. The plates were then incubated with titrated mouse immune sera and normal (control) sera at room temperature for 4 h. Finally the bound peptide-specific antibodies were detected by use of anti-mouse HRP-labelled immunoglobulins (Dako A/S, Denmark) essentially as previously described [Ovod, V. A. *et al.*, AIDS 6 (1992) 25-34].

For western blotting, tissue homogenates, EBV transformed B-cells or PHA-activated T-cells were boiled for 10 minutes in 2x sample buffer (for tissue homogenates: 100 microliters of homogenate mixed with 100 microliters of sample buffer; for cells: one million cells/100 ml of buffer) and analyzed in western blotting as described in Ovod, V. A. *et al., supra*.

The antisera so produced reacted with the AIR-1-protein low amount in normal fetal spleen, thymus and lymphonode as well as, in EBV-transformed B-cells and in PHA-activated T-cells. In the ELISA assay towards the immunogenic peptides, all four mice gave a strong reactivity towards the peptide used for the immunization. In the western blotting analysis using either

the tissue homogenates or stimulated T-cells or established B-cells, a strong band of approx. 60 kD molecular weight was seen in fetal liver (Fig. 6), while weaker bands of the same size were seen in the other samples.

Example 5

Identification of the expression of APECED in thymus and other lymphoid organs

mRNA *in situ* hybridization and immunohistochemistry were used to identify APECED-expressing cells in various normal fetal and adult human tissues. Thymus samples were obtained in conjunction of corrective surgery from cardiac patients aged 2-19 years. Other tissue samples were obtained from surgical biopsy or from autopsy material. This was approved by Hospital Ethics Committees at Tampere University Hospital and Helsinki University Central Hospital. The tissue materials were stored frozen or formaldehyde fixed and paraffin embedded until used.

For mRNA *in situ* hybridization, three cDNA fragments for riboprobes were amplified by RT-PCR from thymus mRNA (Clontech) with primer pairs: 5'-ATG GCG ACG GAC GCG GCG CTA CGC-'3 (seq. id. no. 27) and 5'-CCT GGA TGT ACT TCT TGG AGC CGC-3' (seq. id. no. 28), 5'-GAG CCC GAG GGG CCG TGG AGG GGA-3' (seq. id. no. 29) and 5'-GGC TGC ACC TCC TGG ACT GTT GCC-3' (seq. id. no. 30), and 5'-GAT CCT GCT CAG GAG ACG TGA CCC-3' (seq. id. no. 31) and 5'-CAC CAG GCA AGG AGA GGC TCC CGG-3' (seq. id. no. 32), designed to amplify fragments spanning nucleotides 137 - 812, 738 - 1185 and 1554 - 2009 of the sequence id. no. 1, respectively. The amplified fragments were subcloned into a pCRII-TOPO vector (Invitrogen).

For in vitro transcription the plasmids were linearized and sense and antisense probes were synthesized with digoxigenin-UTP as described (Boehringer Mannheim Nonradioactive *in situ* Hybridization Application Manual). Labeled probes were purified with MicroSpinG-50 columns (Pharmacia Biotech). The pretreatment and hybridization of formaldehyde fixed, paraffin embedded tissue sections were performed as described by H. Breitschopf and G. Sucharek. (Boehringer Mannheim Nonradioactive *in situ* Hybridization Application Manual, Detection of mRNA on paraffin embedded material of the central nervous system with DIG-labeled RNA probes, pp 136-138.)

For the preparation of antibodies to the AIR protein, the APECED cDNA (sequences 137 - 1774 of sequence id. no. 1) containing a full-coding region was amplified from Marathon human thymus cDNA (Clontech) with primers ExF and ExR2. The primer sequences for ExF and ExR2 were 5'-CCA CCC CAT GGC GAC GGA CG-3' (sequence id. no. 33) and 5'-GGA ATT CGG AGG GGA AGG GGG CCG CCG GA-3' (sequence id. no. 34). The amplified cDNA was digested with NcoI and EcoRI and cloned (pHPAIRE) into pET32a vector (Novagen). The protein was expressed in *E. coli* and purified by His-tag as described by manufacturer (QiaExpress Type IV Kit, Cat No 32149, Qiagen, USA).

To obtain murine polyclonal antibodies, Balb/c mice were immunised essentially as described in Example 4 using 100 micrograms of the bacterially expressed AIR protein with booster doses of 25 and 25 micrograms.

Japanese white rabbits were immunised with a synthetic peptide representing amino acids 526-545 (DGILQWAIQSMARPAAPFPS, sequence id. no. 36) of sequence id. no. 2. The specificities of the antisera were checked with ELISA and Western blotting using standard procedures.

For immunocytochemistry, frozen sections of tissue samples were fixed for 20 min in 4% paraformaldehyde. The AIR antibody (rabbit or mouse) in an appropriate dilution was incubated for 30 min at 37°C, with a biotin conjugated anti-mouse or anti–rabbit secondary antidody (Vector, CA, USA). The biotinylated antibody was revealed by incubating with Texas Red-avidin (Vector, CA, USA) for 30 min at 37°C.

With in situ hybridization, a positive signal was seen in a few cells in thymus medulla (Fig. 7A). The APECED *in situ* -positive cells were infrequent and scattered as single cells in the medulla, but occasionally one or two APECED-expressing cells were seen adjacent to or buried into the Hassal's corpuscles that represent conglomerates of medullary epithelial cells. In the positive cells, APECED mRNA was predominantly localized in the cell nucleus. In human adult lymph node tissues, infrequent cells expressed APECED mRNA in the medulla and occasionally in the paracortical region, too (Fig. 7B) No hybridization signal was seen in the germinal centers.

Immunohistochemistry with mouse and rabbit polyclonal antisera to the AIR protein showed strong reactivity with selected cells in thymus medulla, lymph nodes and fetal liver (Fig. 7C and 7D) The comparison of the reaction

pattern obtained by immunohistochemistry to that obtained by in situ hybridization clearly established that specific, rare cells in thymus medulla and lymph node medulla and paracortex express APECED mRNA and the AIR protein. By either method, neither mRNA nor protein was detected in other adult tissues studied, including the target organs for tissue destruction in APECED (adrenal glands, parathyroid glands, gonads). In human fetal tissues, APECED positive cells were seen, although extremely infrequently, in the stroma of placental chorionic villi and in the sinusoidal area of the liver. In the fetal liver, the APECED positive cells were often localized pairwise like mirror images, suggesting that the cells were undergoing mitosis. Rare APECED expressing cells were also found in fetal thymus but the expression was not observed in other fetal tissues.

At the subcellular level, the AIR protein localized in small nuclear dots in the adult thymus, giving a characteristic speckled pattern (Fig. 7C; and Fig. 8A and 8B), but localized in the cytoplasm of cells in lymph nodes. In the rare positive cells in fetal liver, many of which were mitotic, the AIR protein was localized in the cytoplasm.

Example 6

Characterization of the phenotype of the APECED positive cells in thymus

Double staining with two antibodies was used to further characterize the cell type expressing APECED gene. In view of the fact that dendritic cells (DC) and thymus epithelium are both involved in the regulation of immune maturation, expression of markers for these cells were studied.

For double immunofluorencence detection the AIR staining was performed as described in example 5 with rabbit anti-AIR serum. The slides were then incubated with a second primary antibody [AE1 (Neomarkers, CA, USA), AE3 (Neomarkers, CA, USA), CD11c (Immunotech, France), or CD83 (Immunotech, France)] in an appropriate dilution for 30 min at 37°C, and the reaction was revealed by incubating with a FITC conjugated secondary anti-mouse antibody (Vector, CA, USA) for 30 min at 37°C.

Antibodies reacting with low molecular weight basic (AE1) or high molecular weight acidic (AE3) cytokeratins stained the thymus in a reticular fashion, and the APECED positive cells were seen either buried into this net or in close apposition with the keratin-positive cells. Confocal microscopy

clearly demonstrated that some of the APECED positive cells were cytokeratin positive while some remained negative (Figure 8A). A co-localization was stronger with AE1 than with AE3. The distribution of epithelial (AE1 positive) and non-epithelial APECED expressing cells varied but in most thymus preparates more than half were epithelial.

Less than half of the APECED expressing cells in thymus stained with markers CD11c and CD83 that react with cells of the monocyte-macrophage-dendritic cell lineage. In most cases, the staining reaction was weak but a few cells showed an intensive staining with the given marker (Fig. 8B). CD83 costained 5 to 40 % of the APECED positive cells. Antibody CD11c, reported to be specific for mature dendritic cells, reacted with up to 5 - 10 % of the APECED positive cells. All APECED positive cells were strongly positive for HLA-DR staining, however (data not sown).

These results suggest that in thymus the APECED gene is in fact expressed in two distinct cell populations, one epithelial and the other non-epithelial. The latter cell type is likely the one also expressing the APECED gene in extrathymic lymphoid tissues.

Example 7

APECED expression in stimulated dendritic cells *in vitro*

To show an APECED expression in dendritic cells derived from peripheral blood monocytes that are DC precursors, these cells were cultured at the presence of cytokines using conditions that are known to lead to the expansion and maturation of dendritic cells.

Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque centrifugation, and adherent cells were separated and cultured in the presence of human recombinant GM-CSF (1000 units/ml) and rhIL-4 (1000 units/ml, both from R&D Systems), as described [Schuler, G. and Romani, N., Adv. Exp. Med. Biol. 417 (1997) 7 - 13]. Cells were further cultured for three days with 1/4 V/V of macrophage conditioned media. Cells were harvested at two days intervals and samples were prepared for RT-PCR. For RT-PCR total RNA was purified from DCs by using a commercial kit from Clontech (USA) (Nucleospin RNA Kit) according to manufacturer´s instructions. An aliquot of RNA was transferred into cDNA with a commercial kit from Pharmacia (Sweden) (First-strand Synthesis Kit) and PCR for this DNA sample was performed. For PCR the fragment was amplified with primers 5'- GAT CCT

GCT CAG GAG ACG TGA CCC-3' (seq. id. no. 31; 1554 -1577 of seq. id. no. 1) and 5'-GGA CTG AGG AAG GAG GTG TCC TTC -3' (seq. id. no. 35; 1818-1841 of seq. id. no. 1) with the following conditions: 35 cycles of 95°C for 1 min., 62°C for 30 sec and 72°C for 1 min. The 1x reaction mix contained 50mM KCl, 10mM Tris-HCl, pH8.3, 1.5mM $MgCl_2$, 0.001% (w/v) gelatin, 0.2mM each of dNTPs, 0.25 U of Dynazyme (Finnzymes, Finland). A fragment of 287bp was detected by 1.5% agarose electrophoresis.

Cytospin preparations were further made for immunohistochemistry.

During this 7 to 10 days culture period approximately half of the cells developed the characteristic veiled morphology of DC and their phenotypic cell markers (CD11cand CD83) corresponded to mature DCs (Figure 9). The APECED expression was studied by RT-PCR and immunocytochemistry at two to three days intervals. In the starting material, i.e. the adherent cell pool from peripheral blood, no APECED expression was found. After seven days of culture in the presence of GM-CSF and IL-4, RT-PCR showed APECED mRNA expression and immunofluorescence showed a few AIR specific nuclear dots. After an additional 3-day-culture with conditioned medium from macrophage cultures a strong speckled pattern of nuclear AIR expression was seen (Figure 9A). The RT-PCR analysis of the mature (10 days) culture confirmed the AIR protein expression.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Kai Krohn et al.
(B) STREET: Iltarusko, Salmentaantie 751
(C) CITY: 36450 Salmentaka
(E) COUNTRY: Finland
(F) POSTAL CODE (ZIP): none

(ii) TITLE OF INVENTION: Novel Gene

(iii) NUMBER OF SEQUENCES: 26

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2036 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:137..1774
(D) OTHER INFORMATION:/product= "AIR-1"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:137..1771
(D) OTHER INFORMATION:/product= "AIR-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Leu Leu His Ala Leu Ala Asp His Asp Val Val Pro Glu Asp Lys Phe
         30                  35                  40

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Gln Glu Thr Leu His Leu Lys Glu Lys Glu Gly Cys Pro Gln Ala Phe
     45                  50                  55

CAC GCC CTC CTG TCC TGG CTG CTG ACC CAG GAC TCC ACA GCC ATC CTG      361
His Ala Leu Leu Ser Trp Leu Leu Thr Gln Asp Ser Thr Ala Ile Leu
 60                  65                  70                  75

GAC TTC TGG AGG GTG CTG TTC AAG GAC TAC AAC CTG GAG CGC TAT GGC      409
Asp Phe Trp Arg Val Leu Phe Lys Asp Tyr Asn Leu Glu Arg Tyr Gly
                 80                  85                  90

CGG CTG CAG CCC ATC CTG GAC AGC TTC CCC AAA GAT GTG GAC CTC AGC      457
Arg Leu Gln Pro Ile Leu Asp Ser Phe Pro Lys Asp Val Asp Leu Ser
             95                  100                 105

CAG CCC CGG AAG GGG AGG AAG CCC CCG GCC GTC CCC AAG GCT TTG GTA      505
Gln Pro Arg Lys Gly Arg Lys Pro Pro Ala Val Pro Lys Ala Leu Val
        110                 115                 120

CCG CCA CCC AGA CTC CCC ACC AAG AGG AAG GCC TCA GAA GAG GCT CGA      553
Pro Pro Pro Arg Leu Pro Thr Lys Arg Lys Ala Ser Glu Glu Ala Arg
    125                 130                 135

GCT GCC GCG CCA GCA GCC CTG ACT CCA AGG GGC ACC GCC AGC CCA GGC      601
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140                 145                 150                 155

TCT CAA CTG AAG GCC AAG CCC CCC AAG AAG CCG GAG AGC AGC GCA GAG      649
Ser Gln Leu Lys Ala Lys Pro Pro Lys Lys Pro Glu Ser Ser Ala Glu
                160                 165                 170

CAG CAG CGC CTT CCA CTC GGG AAC GGG ATT CAG ACC ATG TCA GCT TCA      697
Gln Gln Arg Leu Pro Leu Gly Asn Gly Ile Gln Thr Met Ser Ala Ser
            175                 180                 185

GTC CAG AGA GCT GTG GCC ATG TCC TCC GGG GAC GTC CCG GGA GCC CGA      745
Val Gln Arg Ala Val Ala Met Ser Ser Gly Asp Val Pro Gly Ala Arg
        190                 195                 200

GGG GCC GTG GAG GGG ATC CTC ATC CAG CAG GTG TTT GAG TCA GGC GGC      793
Gly Ala Val Glu Gly Ile Leu Ile Gln Gln Val Phe Glu Ser Gly Gly
    205                 210                 215

TCC AAG AAG TGC ATC CAG GTT GGC GGG GAG TTC TAC ACT CCC AGC AAG      841
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SUBSTITUTE SHEET (RULE 26)

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            255                 260                 265

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        270                 275                 280

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    285                 290                 295


GCC GTG TGT CGG GAC GGC GGG GAG CTC ATC TGC TGT GAC GGC TGC CCT    1081
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300                 305                 310                 315

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                320                 325                 330

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            335                 340                 345

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Val Gln Pro Arg Ala Glu Glu Pro Arg Pro Gln Glu Pro Pro Val Glu
        350                 355                 360

ACC CCG CTC CCC CCG GGG CTT AGG TCG GCG GGA GAG GAG GTA AGA GGT    1273
Thr Pro Leu Pro Pro Gly Leu Arg Ser Ala Gly Glu Glu Val Arg Gly
    365                 370                 375

CCA CCT GGG GAA CCC CTA GCC GGC ATG GAC ACG ACT CTT GTC TAC AAG    1321
Pro Pro Gly Glu Pro Leu Ala Gly Met Asp Thr Thr Leu Val Tyr Lys
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CAC CTG CCG GCT CCG CCT TCT GCA GCC CCG CTG CCA GGG CTG GAC TCC    1369
His Leu Pro Ala Pro Pro Ser Ala Ala Pro Leu Pro Gly Leu Asp Ser
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Ser Ala Leu His Pro Leu Leu Cys Val Gly Pro Glu Gly Gln Gln Asn
            415                 420                 425

CTG GCT CCT GGT GCG CGT TGC GGG GTG TGC GGA GAT GGT ACG GAC GTG    1465
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SUBSTITUTE SHEET (RULE 26)

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ACC TTC GAT GGC ATC CTG CAG TGG GCC ATC CAG AGC ATG GCC CGT CCG    1753
Thr Phe Asp Gly Ile Leu Gln Trp Ala Ile Gln Ser Met Ala Arg Pro
    525                 530                 535

GCG GCC CCC TTC CCC TCC TGA CCCCAGATGG CCGGGACATG CAGCTCTGAT       1804
Ala Ala Pro Phe Pro Ser  *
540                 545

GAGAGAGTGC TGAGAAGGAC ACCTCCTTCC TCAGTCCTGG AAGCCGGCCG GCTGGGATCA  1864

AGAAGGGGAC AGCGCCACCT CTTGTCAGTG CTCGGCTGTA AACAGCTCTG TGTTTCTGGG  1924

GACACCAGCC ATCATGTGCC TGGAAATTAA ACCCTGCCCC ACTTCTCTAC TCTGGAAGTC  1984

CCCGGGAGCC TCTCCTTGCC TGGTGACCTA CTAAAAATAT AAAAATTAGC TG          2036
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 545 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Ala Asp His Asp Val Val Pro Glu Asp Lys Phe Gln Glu Thr Leu His
        35                  40                  45
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SUBSTITUTE SHEET (RULE 26)

Leu Lys Glu Lys Glu Gly Cys Pro Gln Ala Phe His Ala Leu Leu Ser
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65 70 75 80

Leu Phe Lys Asp Tyr Asn Leu Glu Arg Tyr Gly Arg Leu Gln Pro Ile
85 90 95

Leu Asp Ser Phe Pro Lys Asp Val Asp Leu Ser Gln Pro Arg Lys Gly
100 105 110

Arg Lys Pro Pro Ala Val Pro Lys Ala Leu Val Pro Pro Pro Arg Leu
115 120 125

Pro Thr Lys Arg Lys Ala Ser Glu Glu Ala Arg Ala Ala Ala Pro Ala
130 135 140

Ala Leu Thr Pro Arg Gly Thr Ala Ser Pro Gly Ser Gln Leu Lys Ala
145 150 155 160

Lys Pro Pro Lys Lys Pro Glu Ser Ser Ala Glu Gln Gln Arg Leu Pro
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180 185 190

Ala Met Ser Ser Gly Asp Val Pro Gly Ala Arg Gly Ala Val Glu Gly
195 200 205

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Gln Val Gly Gly Glu Phe Tyr Thr Pro Ser Lys Phe Glu Asp Ser Gly
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275 280 285

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    370                 375                 380

Leu Ala Gly Met Asp Thr Thr Leu Val Tyr Lys His Leu Pro Ala Pro
385                 390                 395                 400

Pro Ser Ala Ala Pro Leu Pro Gly Leu Asp Ser Ser Ala Leu His Pro
                405                 410                 415

Leu Leu Cys Val Gly Pro Glu Gly Gln Gln Asn Leu Ala Pro Gly Ala
            420                 425                 430

Arg Cys Gly Val Cys Gly Asp Gly Thr Asp Val Leu Arg Cys Thr His
        435                 440                 445

Cys Ala Ala Ala Phe His Trp Arg Cys His Phe Pro Ala Gly Thr Ser
    450                 455                 460

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465                 470                 475                 480

Pro Ala Pro Val Glu Gly Val Leu Ala Pro Ser Pro Ala Arg Leu Ala
                485                 490                 495

Pro Gly Pro Ala Lys Asp Asp Thr Ala Ser His Glu Pro Ala Leu His
            500                 505                 510

Arg Asp Asp Leu Glu Ser Leu Leu Ser Glu His Thr Phe Asp Gly Ile
        515                 520                 525

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Ser  *
545
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1545 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

SUBSTITUTE SHEET (RULE 26)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:237..1283
(D) OTHER INFORMATION:/product= "AIR-2"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:237..1280
(D) OTHER INFORMATION:/product= "AIR-2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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GCAGGTCGGG AGAGACCTCC CTGGGCCTGG CCCCACTGCC CTGTGAGGAA GGGTTC       236

ATG TGG TTG GTG TAC AGT TCC GGG GCC CCT GGA ACG CAG CAG CCT GCA      284
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 1               5                  10                  15

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            20                  25                  30

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Trp Arg Pro Asp Gly Trp Gly Thr Gly Gly Gln Gly Arg Ile Ser Gly
        35                  40                  45

CCT GGC AGC ATG GGA GCA GGG CAG AGA CTG GGG AGT TCA GGT ACC CAG      428
Pro Gly Ser Met Gly Ala Gly Gln Arg Leu Gly Ser Ser Gly Thr Gln
    50                  55                  60

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65                  70                  75                  80

GTG CTG CAC CCC AGC CCA GTC TGC ATG GGC GTC TCT TGC CTG TGC CAG      524
Val Leu His Pro Ser Pro Val Cys Met Gly Val Ser Cys Leu Cys Gln
                85                  90                  95

AAG AAT GAG GAC GAG TGT GCC GTG TGT CGG GAC GGC GGG GAG CTC ATC      572
Lys Asn Glu Asp Glu Cys Ala Val Cys Arg Asp Gly Gly Glu Leu Ile
            100                 105                 110

TGC TGT GAC GGC TGC CCT CGG GCC TTC CAC CTG GCC TGC CTG TCC CCT      620
Cys Cys Asp Gly Cys Pro Arg Ala Phe His Leu Ala Cys Leu Ser Pro
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CAG GCA ACA GTC CAG GAG GTG CAG CCC CGG GCA GAG GAG CCC CGG CCC      716
Gln Ala Thr Val Gln Glu Val Gln Pro Arg Ala Glu Glu Pro Arg Pro
145                 150                 155                 160

CAG GAG CCA CCC GTG GAG ACC CCG CTC CCC CCG GGG CTT AGG TCG GCG      764
Gln Glu Pro Pro Val Glu Thr Pro Leu Pro Pro Gly Leu Arg Ser Ala
                165                 170                 175

GGA GAG GAG GTA AGA GGT CCA CCT GGG GAA CCC CTA GCC GGC ATG GAC      812
Gly Glu Glu Val Arg Gly Pro Pro Gly Glu Pro Leu Ala Gly Met Asp
            180                 185                 190

ACG ACT CTT GTC TAC AAG CAC CTG CCG GCT CCG CCT TCT GCA GCC CCG      860
Thr Thr Leu Val Tyr Lys His Leu Pro Ala Pro Pro Ser Ala Ala Pro
        195                 200                 205

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    210                 215                 220

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225                 230                 235                 240

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Gly Asp Gly Thr Asp Val Leu Arg Cys Thr His Cys Ala Ala Ala Phe
                245                 250                 255

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His Trp Arg Cys His Phe Pro Ala Gly Thr Ser Arg Pro Gly Thr Gly
            260                 265                 270

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        275                 280                 285

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    290                 295                 300

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Asp Asp Thr Ala Ser His Glu Pro Ala Leu His Arg Asp Asp Leu Glu
305                 310                 315                 320

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SUBSTITUTE SHEET (RULE 26)

CCGGGACATG CAGCTCTGAT GAGAGAGTGC TGAGAAGGAC ACCTCCTTCC TCAGTCCTGG 1353

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ACTTCTCTAC TCTGGAAGTC CCCGGGAGCC TCTCCTTGCC TGGTGACCTA CTAAAAATAT 1533

AAAAATTAGC TG 1545

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 348 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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35 40 45

Pro Gly Ser Met Gly Ala Gly Gln Arg Leu Gly Ser Ser Gly Thr Gln
50 55 60

Arg Cys Cys Trp Gly Ser Cys Phe Gly Lys Glu Val Ala Leu Arg Arg
65 70 75 80

Val Leu His Pro Ser Pro Val Cys Met Gly Val Ser Cys Leu Cys Gln
85 90 95

Lys Asn Glu Asp Glu Cys Ala Val Cys Arg Asp Gly Gly Glu Leu Ile
100 105 110

Cys Cys Asp Gly Cys Pro Arg Ala Phe His Leu Ala Cys Leu Ser Pro
115 120 125

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        195                 200                 205

Leu Pro Gly Leu Asp Ser Ser Ala Leu His Pro Leu Leu Cys Val Gly
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Pro Glu Gly Gln Gln Asn Leu Ala Pro Gly Ala Arg Cys Gly Val Cys
225                 230                 235                 240

Gly Asp Gly Thr Asp Val Leu Arg Cys Thr His Cys Ala Ala Ala Phe
                245                 250                 255

His Trp Arg Cys His Phe Pro Ala Gly Thr Ser Arg Pro Gly Thr Gly
            260                 265                 270

Leu Arg Cys Arg Ser Cys Ser Gly Asp Val Thr Pro Ala Pro Val Glu
        275                 280                 285

Gly Val Leu Ala Pro Ser Pro Ala Arg Leu Ala Pro Gly Pro Ala Lys
    290                 295                 300

Asp Asp Thr Ala Ser His Glu Pro Ala Leu His Arg Asp Asp Leu Glu
305                 310                 315                 320

Ser Leu Leu Ser Glu His Thr Phe Asp Gly Ile Leu Gln Trp Ala Ile
                325                 330                 335

Gln Ser Met Ala Arg Pro Ala Ala Pro Phe Pro Ser  *
            340                 345
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1463 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:237..1001
(D) OTHER INFORMATION:/product= "AIR-3"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION:237..998
(D) OTHER INFORMATION:/product= "AIR-3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
AGAGAAAGTG AGGTCTTCTC AGGCTCTTAA GAGCATGGCG TTTGGTCCAG GCTGTACCCG    60

CTGCTCTCAG CTGGGCCCGT GGGTGGGCCG GGCGCCCCTG CTATAGCCAG GAGGTCAAGG   120

ATCCACTGGG AATGCCATGC TCATCTTTCG TCCCCAGCAT GGTTTCTTAA TGGGGTAGAA   180

GCAGGTCGGG AGAGACCTCC CTGGGCCTGG CCCCACTGCC CTGTGAGGAA GGGTTC       236

ATG TGG TTG GTG TAC AGT TCC GGG GCC CCT GGA ACG CAG CAG CCT GCA      284
Met Trp Leu Val Tyr Ser Ser Gly Ala Pro Gly Thr Gln Gln Pro Ala
 1               5                  10                  15

AGA AAC CGG GTT TTC TTC CCA ATA GGG ATG GCC CCG GGG GGT GTC TGT      332
Arg Asn Arg Val Phe Phe Pro Ile Gly Met Ala Pro Gly Gly Val Cys
            20                  25                  30

TGG AGA CCA GAT GGA TGG GGA ACA GGT GGT CAG GGC AGA ATT TCA GGC      380
Trp Arg Pro Asp Gly Trp Gly Thr Gly Gly Gln Gly Arg Ile Ser Gly
        35                  40                  45

CCT GGC AGC ATG GGA GCA GGG CAG AGA CTG GGG AGT TCA GGT ACC CAG      428
Pro Gly Ser Met Gly Ala Gly Gln Arg Leu Gly Ser Ser Gly Thr Gln
    50                  55                  60

AGA TGC TGC TGG GGG AGC TGT TTT GGG AAG GAG GTG GCT CTC AGG AGG      476
Arg Cys Cys Trp Gly Ser Cys Phe Gly Lys Glu Val Ala Leu Arg Arg
65                  70                  75                  80

GTG CTG CAC CCC AGC CCA GTC TGC ATG GGC GTC TCT TGC CTG TGC CAG      524
Val Leu His Pro Ser Pro Val Cys Met Gly Val Ser Cys Leu Cys Gln
                85                  90                  95

AAG AAT GAG GAC GAG TGT GCC GTG TGT CGG GAC GGC GGG GAG CTC ATC      572
Lys Asn Glu Asp Glu Cys Ala Val Cys Arg Asp Gly Gly Glu Leu Ile
            100                 105                 110

TGC TGT GAC GGC TGC CCT CGG GCC TTC CAC CTG GCC TGC CTG TCC CCT      620
Cys Cys Asp Gly Cys Pro Arg Ala Phe His Leu Ala Cys Leu Ser Pro
        115                 120                 125

CCG CTC CGG GAG ATC CCC AGT GGG ACC TGG AGG TGC TCC AGC TGC CTG      668
Pro Leu Arg Glu Ile Pro Ser Gly Thr Trp Arg Cys Ser Ser Cys Leu
    130                 135                 140

CAG GCA ACA GTC CAG GAG GTG CAG CCC CGG GCA GAG GAG CCC CGG CCC      716
Gln Ala Thr Val Gln Glu Val Gln Pro Arg Ala Glu Glu Pro Arg Pro
145                 150                 155                 160

CAG GAG CCA CCC GTG GAG ACC CCG CTC CCC CCG GGG CTT AGG TCG GCG      764
Gln Glu Pro Pro Val Glu Thr Pro Leu Pro Pro Gly Leu Arg Ser Ala
                165                 170                 175
```

```
GGA GAG GAG CCC CGC TGC CAG GGC TGG ACT CCT CGG CCC TGC ACC CCC      812
Gly Glu Glu Pro Arg Cys Gln Gly Trp Thr Pro Arg Pro Cys Thr Pro
            180                 185                 190

TAC TGT GTG TGG GTC CTG AGG GTC AGC AGA ACC TGG CTC CTG GTG CGC      860
Tyr Cys Val Trp Val Leu Arg Val Ser Arg Thr Trp Leu Leu Val Arg
        195                 200                 205

GTT GCG GGG TGT GCG GAG ATG GTA CGG ACG TGC TGC GGT GTA CTC ACT      908
Val Ala Gly Cys Ala Glu Met Val Arg Thr Cys Cys Gly Val Leu Thr
    210                 215                 220

GCG CCG CTG CCT TCC ACT GGC GCT GCC ACT TCC CAG CCG GCA CCT CCC      956
Ala Pro Leu Pro Ser Thr Gly Ala Ala Thr Ser Gln Pro Ala Pro Pro
225                 230                 235                 240

GGC CCG GGA CGG GCC TGC GCT GCA GAT CCT GCT CAG GAG ACG TGA          1001
Gly Pro Gly Arg Ala Cys Ala Ala Asp Pro Ala Gln Glu Thr  *
                245                 250                 255

CCCCAGCCCC TGTGGAGGGG GTGCTGGCCC CCAGCCCCGC CCGCCTGGCC CCTGGGCCTG  1061

CCAAGGATGA CACTGCCAGT CACGAGCCCG CTCTGCACAG GGATGACCTG GAGTCCCTTC  1121

TGAGCGAGCA CACCTTCGAT GGCATCCTGC AGTGGGCCAT CCAGAGCATG GCCCGTCCGG  1181

CGGCCCCCTT CCCCTCCTGA CCCCAGATGG CCGGGACATG CAGCTCTGAT GAGAGAGTGC  1241

TGAGAAGGAC ACCTCCTTCC TCAGTCCTGG AAGCCGGCCG GCTGGGATCA AGAAGGGGAC  1301

AGCGCCACCT CTTGTCAGTG CTCGGCTGTA AACAGCTCTG TGTTTCTGGG GACACCAGCC  1361

ATCATGTGCC TGGAAATTAA ACCCTGCCCC ACTTCTCTAC TCTGGAAGTC CCCGGGAGCC  1421

TCTCCTTGCC TGGTGACCTA CTAAAAATAT AAAAATTAGC TG                      1463
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 254 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Trp Leu Val Tyr Ser Ser Gly Ala Pro Gly Thr Gln Gln Pro Ala
  1               5                  10                  15

Arg Asn Arg Val Phe Phe Pro Ile Gly Met Ala Pro Gly Gly Val Cys
            20                  25                  30

Trp Arg Pro Asp Gly Trp Gly Thr Gly Gly Gln Gly Arg Ile Ser Gly
        35                  40                  45
```

```
Pro Gly Ser Met Gly Ala Gly Gln Arg Leu Gly Ser Ser Gly Thr Gln
     50                  55                  60

Arg Cys Cys Trp Gly Ser Cys Phe Gly Lys Glu Val Ala Leu Arg Arg
 65                  70                  75                  80

Val Leu His Pro Ser Pro Val Cys Met Gly Val Ser Cys Leu Cys Gln
                 85                  90                  95

Lys Asn Glu Asp Glu Cys Ala Val Cys Arg Asp Gly Gly Glu Leu Ile
            100                 105                 110

Cys Cys Asp Gly Cys Pro Arg Ala Phe His Leu Ala Cys Leu Ser Pro
        115                 120                 125

Pro Leu Arg Glu Ile Pro Ser Gly Thr Trp Arg Cys Ser Ser Cys Leu
    130                 135                 140

Gln Ala Thr Val Gln Glu Val Gln Pro Arg Ala Glu Glu Pro Arg Pro
145                 150                 155                 160

Gln Glu Pro Pro Val Glu Thr Pro Leu Pro Pro Gly Leu Arg Ser Ala
                165                 170                 175

Gly Glu Glu Pro Arg Cys Gln Gly Trp Thr Pro Arg Pro Cys Thr Pro
            180                 185                 190

Tyr Cys Val Trp Val Leu Arg Val Ser Arg Thr Trp Leu Leu Val Arg
        195                 200                 205

Val Ala Gly Cys Ala Glu Met Val Arg Thr Cys Cys Gly Val Leu Thr
    210                 215                 220

Ala Pro Leu Pro Ser Thr Gly Ala Ala Thr Ser Gln Pro Ala Pro Pro
225                 230                 235                 240

Gly Pro Gly Arg Ala Cys Ala Ala Asp Pro Ala Gln Glu Thr  *
                245                 250                 255
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GATGACACTG CCAGTCACGA 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTTCCCGAGT GGAAGGCGCT GC 22

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AGGGGACAGG CAGGCCAGGT 20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GAGTTCAGGT ACCCAGAGAT GCTG 24

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CTCGCTCAGA AGGGACTCCA 20

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGATTCAGAC CATGTCAGCT TCA 23

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GTGCTGTTCA AGGACTACAA C 21

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TGGATGAGGA TCCCCTCCAC G 21

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CCATCCTAAT ACGACTCACT ATAGGGC 27

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(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TGCAGGCTGT GGGAACTCCA 20

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AGAAAAAGAG CTGTACCCTG TG 22

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TGCAAGGAAG AGGGGCGTCA GC 22

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TCCACCACAA GCCGAGGAGA T 21

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(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

ACGGGCTCCT CAAACACCAC T 21

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TGGAGATGGG CAGGCCGCAG GGTG 24

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CAGTCCAGCT GGGCTGAGCA GGTG 24

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GCGGCTCCAA GAAGTGCATC CAGG 24

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(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CTCCACCCTG CAAGGAAGAG GGGC 24

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Thr Leu His Leu Lys Glu Lys Glu Gly Cys Pro Gln Ala Phe His
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Gly Lys Asn Lys Ala Arg Ser Ser Ser Gly Pro Lys Pro Leu Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

ATGGCGACGG ACGCGGCGCT ACGC

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CCTGGATGTA CTTCTTGGAG CCGC

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GAGCCCGAGG GGCCGTGGAG GGGA

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GGCTGCACCT CCTGGACTGT TGCC

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GATCCTGCTC AGGAGACGTG ACCC

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CACCAGGCAA GGAGAGGCTC CCGG

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CCACCCCATG GCGACGGACG

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GGAATTCGGA GGGGAAGGGG GCCGCCGGA

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GGACTGAGGA AGGAGGTGTC CTTC

2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Asp	Gly	Ile	Leu	Gln	Trp	Ala	Ile	Gln	Ser	Met	Ala	Arg	Pro	Ala	Ala	Pro	Phe	Pro	Ser
1				5					10						15				20

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Claims

1. An isolated DNA sequence characterized by comprising the sequence id. no. 1 or a functional fragment or variant thereof encoding a protein having the same functional activity, or an functionally equivalent isolated DNA sequence hybridizable thereto.

2. An isolated DNA sequence according to claim 1, characterized in that it is associated with diseases related to immune maturation and regulation of immune response towards self and nonself, such as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED).

3. An isolated DNA-sequence according to claim 1 or 2, characterized in that it includes a gene defect responsible for APECED.

4. A DNA sequence according to claim 1, characterized by having the sequence according to sequence id. no 1 or a functional fragment thereof having the sequence according to sequence id. no 3 or sequence id. no 5.

5. A protein characterized by comprising the amino acid sequence id. no. 2 or a functional fragment or variant thereof having the same functional properties.

6. A protein according to claim 5, characterized in that it is associated diseases related to immune maturation and regulation of immune response towards self and nonself, such as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED).

7. A protein according to claim 5 or 6, characterized by having the amino acid sequence id. no. 2, or a functional fragment thereof having the sequence according to sequence id. no. 4, or a functional fragment thereof having the sequence according to sequence id. no 6.

8. A protein according to any of claims 5 to 7, characterized by having distinct structural motifs, including the PHD finger motif (PHD), the LXXLL motif (L), proline-rich region (PRR), and cystein-rich region (CRR).

9. A method for the diagnosis of diseases related to immune maturation and regulation of immune response towards self and nonself, characterized by detecting in a biological specimen the presence of a DNA sequence comprising the sequence id. no. 1 or a functional fragment or variant thereof encoding a protein having the same functional activity, or a functionally equivalent isolated DNA-sequence hybridizable thereto.

10. A method according to claim 9, characterized in that the DNA sequence is associated with APECED.

11. A method according to claim 9 or 10, characterized in that the DNA sequence includes a gene defect responsible for APECED.

12. A method according to claim 11, characterized in that the gene defect to be detected includes a "C" to "T" transition resulting in the "Arg" to "Stop" nonsense mutation at amino acid position 257 and/or a "A" to "G" transversion resulting in the "Lys" to "Glu" missense mutation at amino acid position 42.

13. A method according to any one of claims 9 to 12, characterized in that DNA techniques are used for the detection.

14. A method according to any one of claims 9 to 13, characterized in that the detection takes advantage of TaqI or another enzyme cleaving at recognition site 5'-TCGA-3' digestion.

15. A method according to any one of claims 9 to 14, characterized in that the disease is autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED).

16. A method for the diagnosis of diseases related to immune maturation and regulation of immune response towards self and nonself, characterized by detecting in a biological specimen the presence or the absence of a protein comprising the sequence id. no. 2, or a functional fragment thereof having the sequence according to sequence id. no. 4, or a functional fragment thereof having the sequence according to sequence id. no. 6.

17. A method according to claim 16, characterized in that the protein is associated with APECED.

18. A method according to claim 16 or 17, characterized in that the disease is autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED).

19. The use of the DNA sequence according to any one of claims 1 to 4 in the diagnosis of diseases related to immune maturation and regulation of immune response towards self and nonself, such as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED).

20. The use of the protein according to any one of claims 5 to 7 in the diagnosis of diseases related to immune maturation and regulation of immune response towards self and nonself, such as autoimmune poly-endocrinopathy-candidiasis-ectodermal dystrophy (APECED).

21. The use of the DNA sequence according to any one of claims 1 to 4 for the preparation of a medicament useful in a gene therapy method of diseases related to immune maturation and regulation of immune response towards self and nonself, such as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED).

22. The use of the DNA sequence according to any one of claims 1 to 4 in the treatment of diseases related to immune maturation and regulation of immune response towards self and nonself, such as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED).

23. Reagents reacting with the DNA sequence according to any one of claims 1 to 4 or the protein of any one of the claims 5 to 8 or with reagents reacting therewith.

24. Reagents according to claim 23, characterized in that they are antibodies.

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DOCOTT"85980560

Human chromosome 21

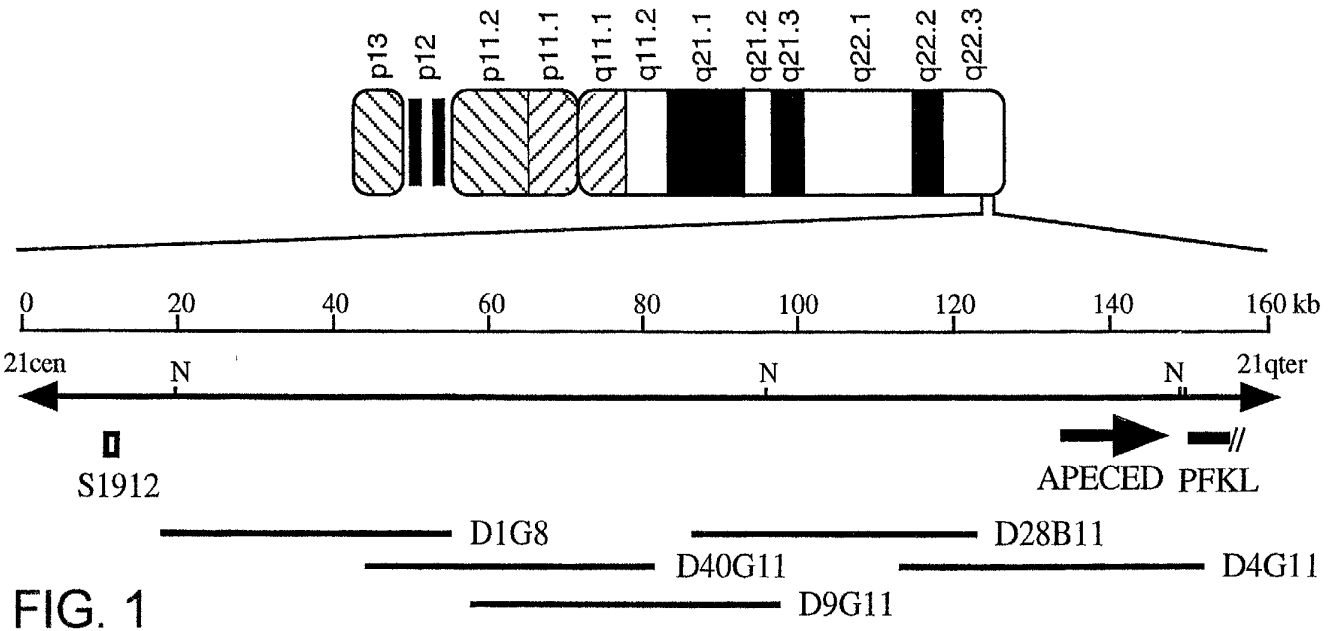

FIG. 1

09/508658

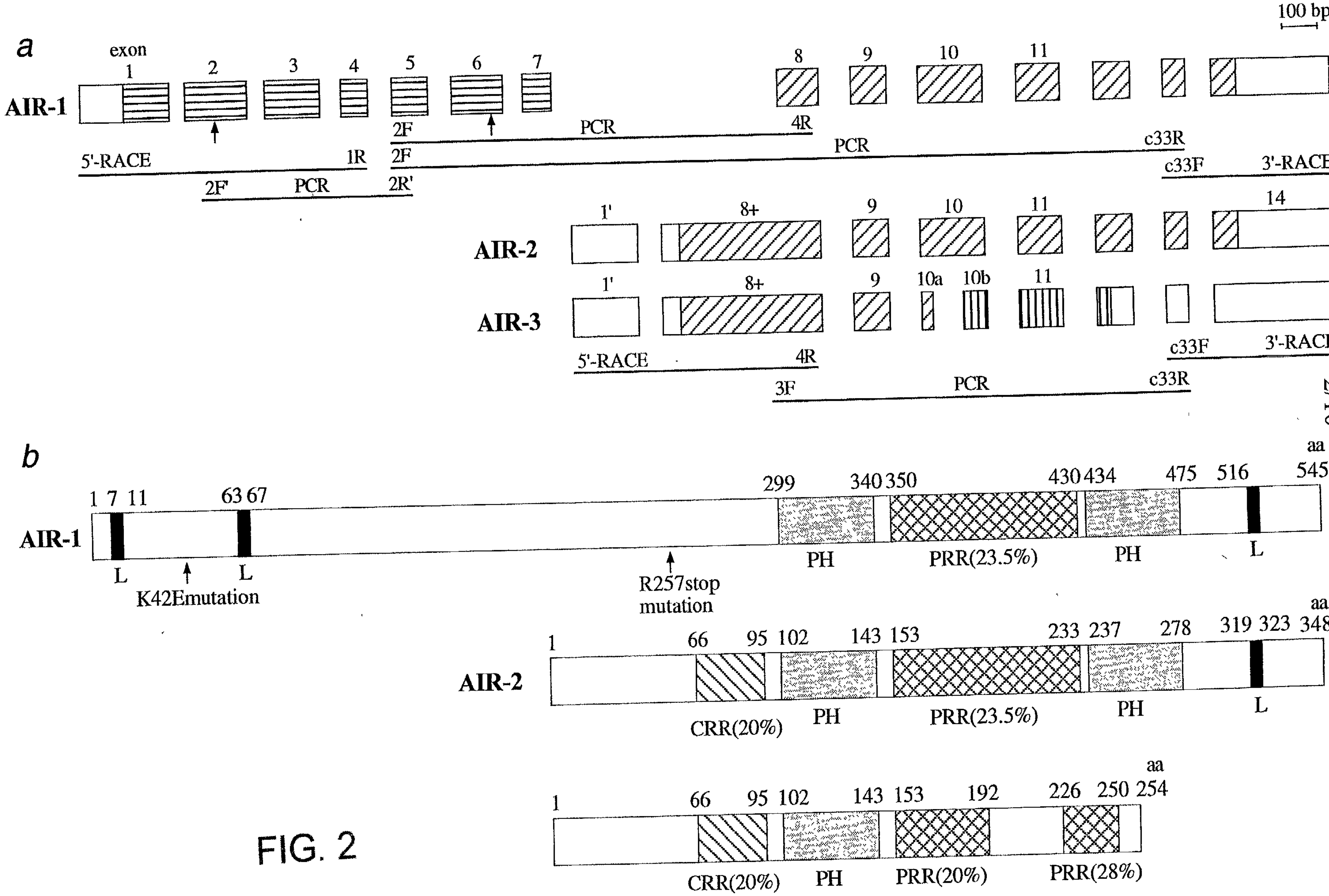
a
100 bp
AIR-1
exon
1
2
3
4
5
6
7
8
9
10
11
5'-RACE
1R
2F
PCR
2R'
2F
PCR
4R
2F
PCR
c33R
c33F
3'-RACE
AIR-2
1'
8+
14
AIR-3
10a
10b
5'-RACE
4R
3F
PCR
c33R
c33F
3'-RACE
b
AIR-1
1 7 11
63 67
299
340 350
430 434
475
516
aa 545
L
K42Emutation
R257stop mutation
PH
PRR(23.5%)
AIR-2
1
66
95 102
143 153
233 237
278
319 323
aa 348
CRR(20%)
192
226
250
aa 254
PRR(20%)
PRR(28%)

FIG. 2

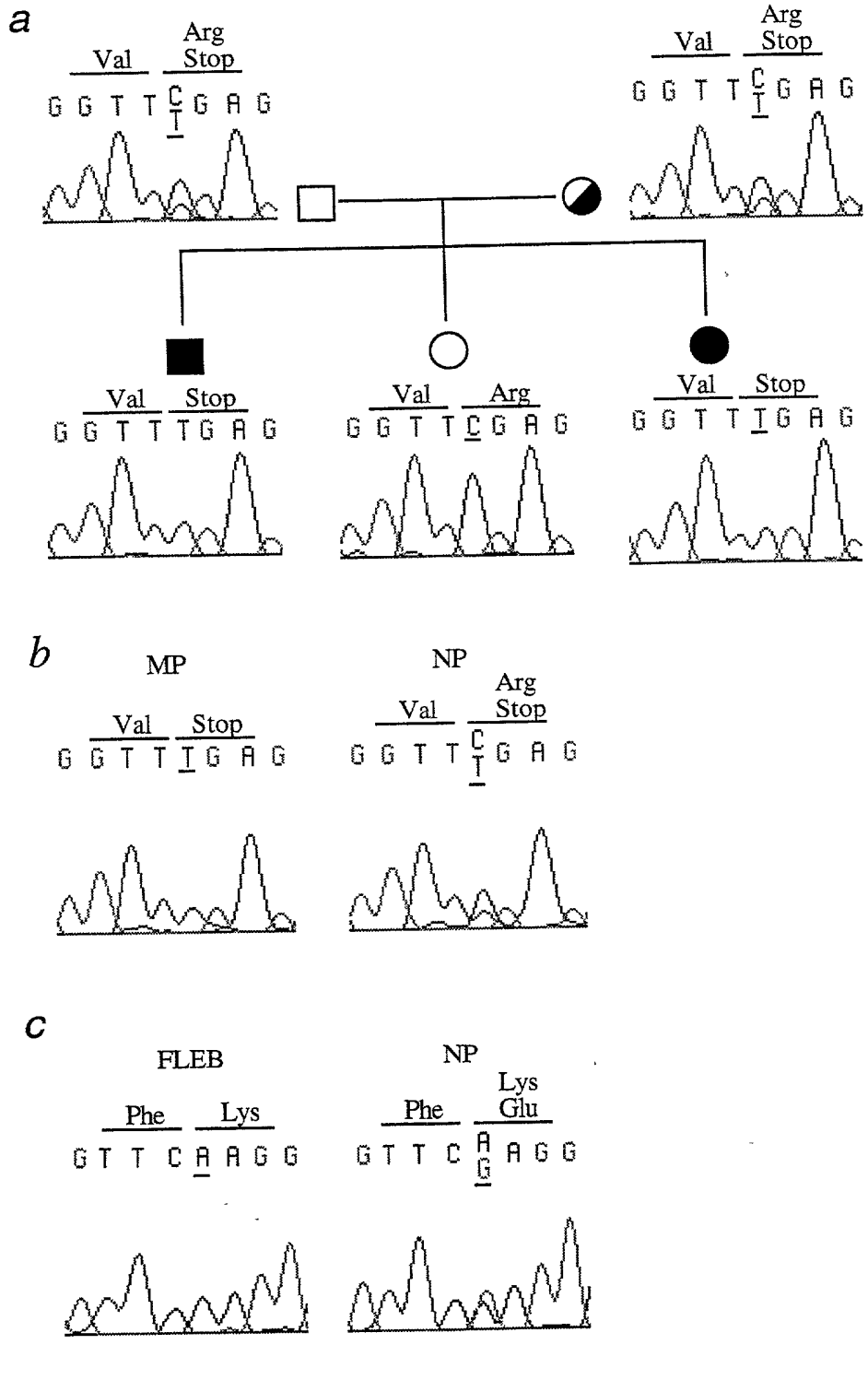
a
Val
Arg
Stop
G G T T C G A G
Val
Arg
Stop
G G T T C G A G
Val
Stop
G G T T T G A G
Val
Arg
G G T T C G A G
Val
Stop
G G T T T G A G
b
MP
Val
Stop
G G T T T G A G
NP
Val
Arg
Stop
G G T T C G A G
c
FLEB
Phe
Lys
G T T C A A G G
NP
Phe
Lys
Glu
G T T C A A G G

FIG. 3

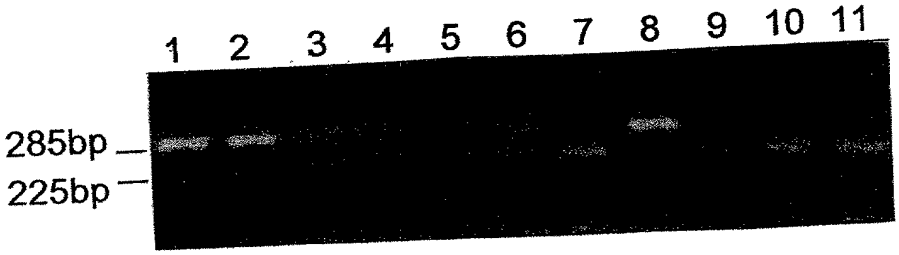

FIG. 4

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```
AIR-1: 299 CAVCRDGGELICCDGCPRAFHLACLSPPLREIPSGTWRCSSC 340
AIR-1: 434 .G..G..TDVLR.TH.AA...WR.HF.AGTSR.GTGL..R.. 475
Mi-2 : 373 .E..QQ...I.L..T....Y.MV..D.DMEKA.E.K.S.PH. 414
Mi-2 : 452 .R..K.........T..SSY.IH..N...P...N.E.L.PR. 493
TIF1 : 791 ....QN.......EK..KV...S.HV.T.TNF...E.I.TF. 832
consensus  C  C        C  C    H  C                C  C
```

FIG. 5

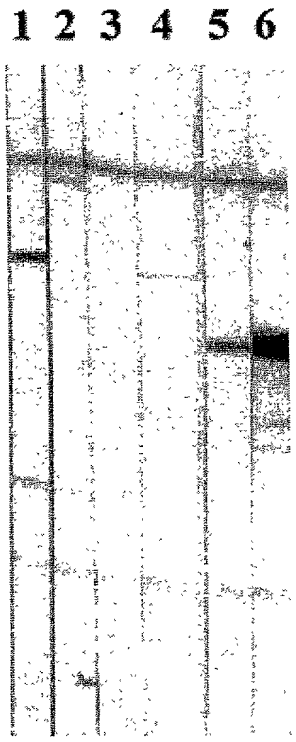

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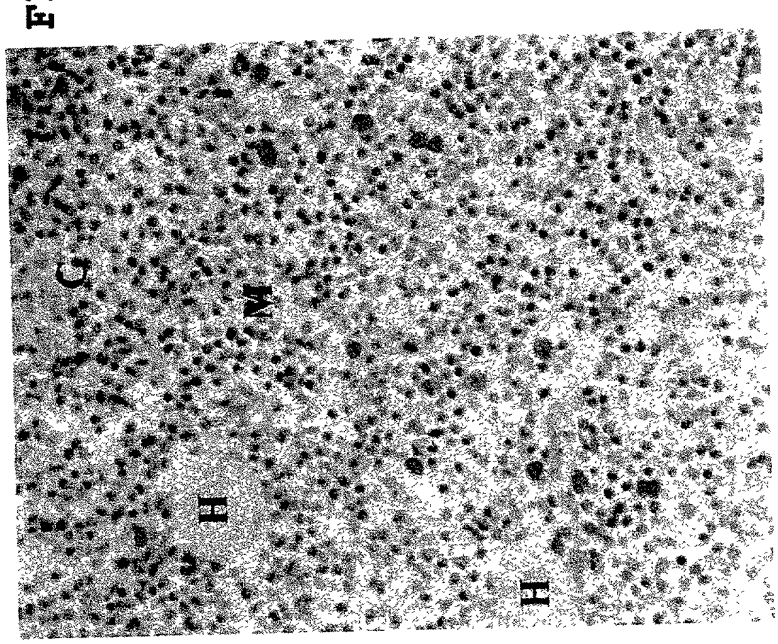
FIG. 7B



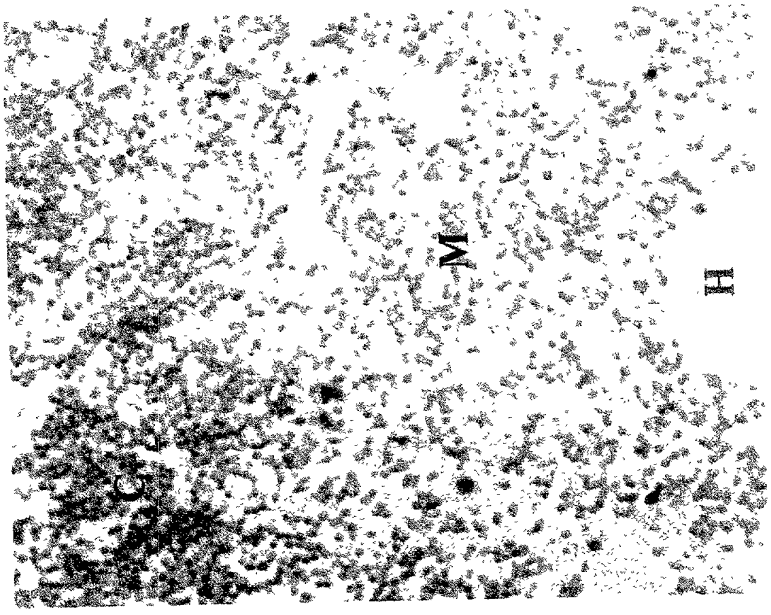


FIG. 7A

09508658 - 090599

FIG. 7D

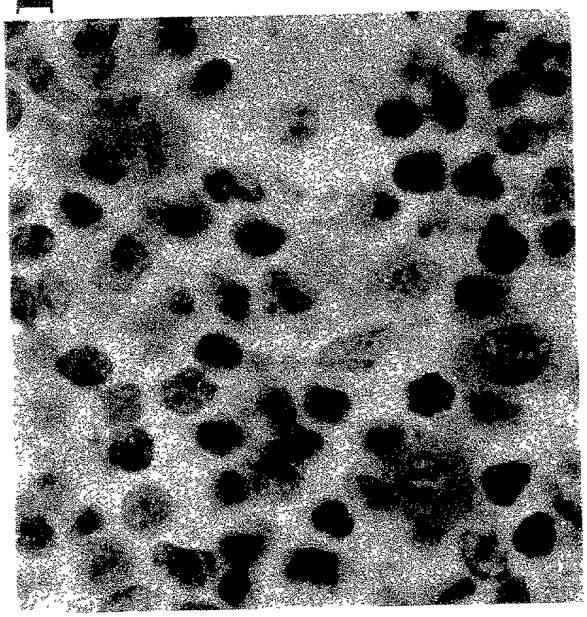
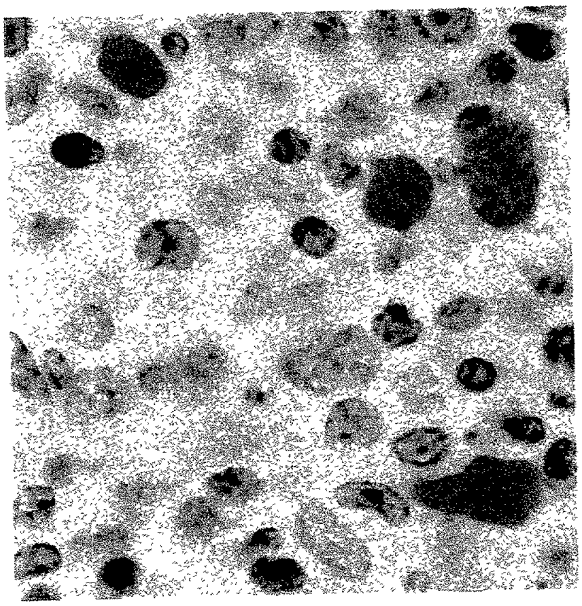


FIG. 7C



09508658 - 112099

FIG. 8A

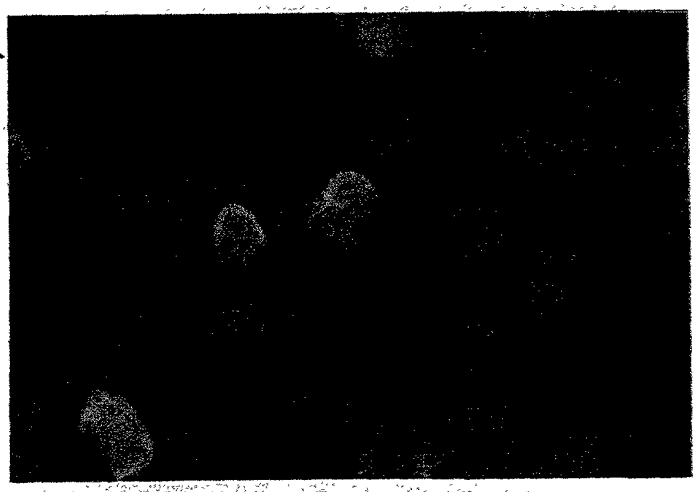
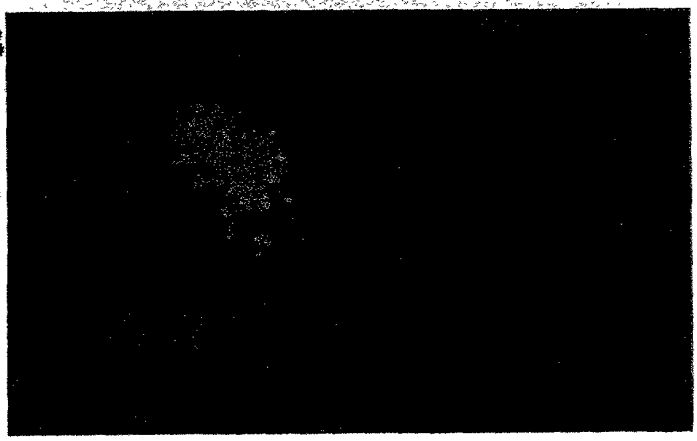


FIG. 8B



SUBSTITUTE SHEET (RULE 26)

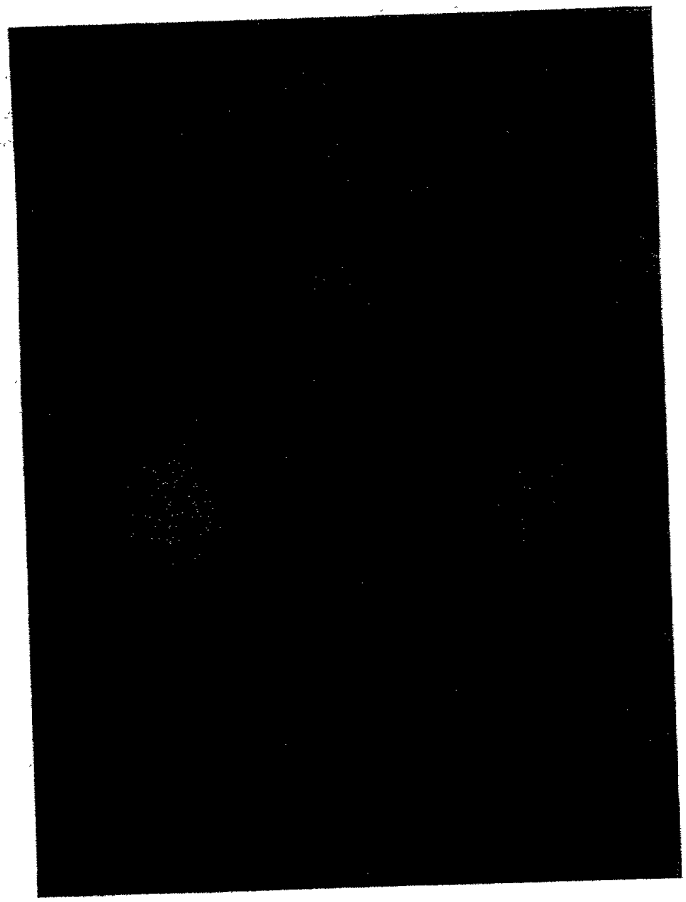


FIG. 9

09508658 - 091500

2971064

Attorney's Docket No. U 012653-9 **PATENT**

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type:

(check one applicable item below)

- ☐ original.
- ☐ design.
- ☐ supplemental.

NOTE If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

- ☒ national stage of PCT.

NOTE If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P

- ☐ divisional.
- ☐ continuation.
- ☐ continuation-in-part (C-I-P).

INVENTORSHIP IDENTIFICATION

WARNING: *If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted*

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

Novel gene defective in apeced and its use

SPECIFICATION IDENTIFICATION

the specification of which

(complete (a), (b) or (c))

(a) ☐ is attached hereto.

(b) ☐ was filed on _____, as ☐ Serial No. 0 /_____ or ☐ Express Mail No., as Serial No. not yet known _____ and was amended on _____ *(if applicable)*.

NOTE Amendments filed after the original papers are deposited with the PTO that contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 CFR 1.67

(c) ☒ was described and claimed in PCT International Application No. PCT/FI98/00749, filed on 23 Sept. 1998 and as amended under PCT Article 19 on _____ *(if any)*.

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56.

(also check the following items, if desired)

☐ and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and

☐ in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 CFR 1.98.

PRIORITY CLAIM (35 U.S.C. § 119(a)–(d))

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)–(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) ☐ no such applications have been filed.

(e) ☒ such applications have been filed as follows.

NOTE Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119(a)-(d)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 USC 119
FI	973762	23 Sept. 1997	☒ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(34 U.S.C. § 119(e))

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER	FILING DATE
_____ /_____	_____
_____ /_____	_____
_____ /_____	_____

CLAIM FOR BENEFIT OF EARLIER US/PCT APPLICATION(S) UNDER 35 U.S.C. 120

☐ The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN PART (C-I-P) APPLICATION.

ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

NOTE. *If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. § 120.*

POWER OF ATTORNEY

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

15

PAUL B WEST, 18947
JOSEPH H HANDELMAN, 26179
JOHN RICHARDS, 31053
JOHN J CHRYSTAL, 26360
RICHARD J STREIT, 25765
ALAN K ROBERTS, 17777
S. DELVALLE GOLDSMITH, 14383
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LAIN C. BAILLIE, 24090
THOMAS F. PETERSON, 24790
RICHARD P. BERG, 28145
JULIAN H. COHEN, 20302
WILLIAM R. EVANS, 25858
JANET I. CORD, 33778
CLIFFORD J. MASS, 30086

(check the following item, if applicable)

☐ Attached, as part of this declaration and power of attorney, is the authorization of the above-named attorney(s) to accept and follow instructions from my representative(s)

SEND CORRESPONDENCE TO

LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023

DIRECT TELEPHONE CALLS TO.
(Name and telephone number)

(212)708-1930

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2

(check proper box(es) for any of the following added page(s) that form a part of this declaration)

☒ **Signature** for fourth and subsequent joint inventors. *Number of pages added* 2

• • •

☐ **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* _____

• • •

☐ **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. *Number of pages added* _____

• • •

☐ Added page for **signature** by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 CFR 1.47)

• • •

☐ Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

☐ Number of pages added _____

• • •

☐ Authorization of attorney(s) to accept and follow instructions from representative.

• • •

(if no further pages form a part of this Declaration, then end this Declaration with this page and check the following item)

☐ This declaration ends with this page.

SIGNATURE(S)

NOTE: Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other documents.

1-00

Full name of sole or first inventor

Kai (GIVEN NAME) (MIDDLE INITIAL OR NAME) Krohn (FAMILY (OR LAST NAME))

Inventor's signature

Date 2.3.2000 **Country of Citizenship** Finland

Residence Salmentaantie 751 FIX

Post Office Address 36450 SALMENTAKA

2-00

Full name of second joint inventor, if any

Maarit (GIVEN NAME) (MIDDLE INITIAL OR NAME) Heino (FAMILY (OR LAST NAME))

Inventor's signature

Date 2.3.2000 **Country of Citizenship** Finland

Residence Kaskitie 6 1 94 FI

Post Office Address 33540 TA

3-00

Full name of third joint inventor, if any

Pärt (GIVEN NAME) (MIDDLE INITIAL OR NAME) Peterson (FAMILY (OR LAST NAME))

Inventor's signature

Date 2.3.2000 **Country of Citizenship** Estonia

Residence Kaskitie 13 F 61 FIX

Post Office Address 33540 TAMPERE

2nd HOUSE US

Attorney's Docket No. U 012653-9

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

4-w

Full name of fourth joint inventor, if any

Hamish (GIVEN NAME) S (MIDDLE INITIAL OR NAME) Scott (FAMILY (OR LAST NAME))

Inventor's signature Hamish Scott

Date 8/10/00 Country of Citizenship Australia

Residence 15 KINGSLEY GARDENS ALX

Post Office Address BRUNSWICK WEST

VICTORIA 3057 AUSTRALIA

Full name of fifth joint inventor, if any

Stylianos (GIVEN NAME) (MIDDLE INITIAL OR NAME) Antonarakis (FAMILY (OR LAST NAME))

Inventor's signature

Date Country of Citizenship US/Greece

Residence

Post Office Address

Full name of sixth joint inventor, if any

Maria (GIVEN NAME) D (MIDDLE INITIAL OR NAME) Lalioti (FAMILY (OR LAST NAME))

Inventor's signature

Date 10-10-00 Country of Citizenship GREECE

Residence 79 STOCKDALE PLACE

Post Office Address EDGBASTON BIRMINGHAM

B15 3XH UK

29406 / US

Attorney's Docket No. U 012653-9

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

4-00

Full name of fourth joint inventor, if any

Hamish | | Scott

GIVEN NAME | MIDDLE INITIAL OR NAME | FAMILY (OR LAST NAME)

Inventor's signature _____

Date _____ Country of Citizenship Australia

Residence _____

Post Office Address _____

5-00

Full name of fifth joint inventor, if any

Stylianos | E. | Antonarakis

GIVEN NAME | MIDDLE INITIAL OR NAME | FAMILY (OR LAST NAME)

Inventor's signature S.E. Antonarakis

Date 29 Sep 00 Country of Citizenship US/Greece

Residence 28 Blvd des Philosophes CH

Post Office Address 1205 Geneva

Switzerland

6-00

Full name of sixth joint inventor, if any

Maria | D. | Lalioti

GIVEN NAME | MIDDLE INITIAL OR NAME | FAMILY (OR LAST NAME)

Inventor's signature [signature]

Date 18-10-00 Country of Citizenship GREECE

Residence 79 STOCKDALE PL. EDGBASTON UK

Post Office Address BIRMINGHAM UK B15 3XH

SIGNATURE(S)

NOTE. Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other documents.

Full name of sole or first inventor

Kai _____ _____ Krohn
(GIVEN NAME) *(MIDDLE INITIAL OR NAME)* *FAMILY (OR LAST NAME)*

Inventor's signature _____

Date _____ **Country of Citizenship** Finland

Residence _____

Post Office Address _____

_____

Full name of second joint inventor, if any

Maarit _____ _____ Heino
(GIVEN NAME) *(MIDDLE INITIAL OR NAME)* *FAMILY (OR LAST NAME)*

Inventor's signature _____

Date _____ **Country of Citizenship** Finland

Residence _____

Post Office Address _____

_____

Full name of third joint inventor, if any

Pärt _____ _____ Peterson
(GIVEN NAME) *(MIDDLE INITIAL OR NAME)* *FAMILY (OR LAST NAME)*

Inventor's signature _____

Date _____ **Country of Citizenship** Estonia

Residence _____

Post Office Address _____

_____

(check proper box(es) for any of the following added page(s) that form a part of this declaration)

☒ **Signature** for fourth and subsequent joint inventors. *Number of pages added* 2

. . .

☐ **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* _____

. . .

☐ **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. *Number of pages added* _____

. . .

☐ Added page for **signature** by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 CFR 1.47)

. . .

☐ Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

☐ Number of pages added _____ .

. . .

☐ Authorization of attorney(s) to accept and follow instructions from representative.

. . .

(if no further pages form a part of this Declaration, then end this Declaration with this page and check the following item)

☐ This declaration ends with this page.

Attorney's Docket No. _____

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

Full name of 7th joint inventor, if any

Nobuyoshi (GIVEN NAME) _____ (MIDDLE INITIAL OR NAME) Shimizu (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship Japan

Residence _____

Post Office Address _____

_____

Full name of 8th joint inventor, if any

Jun (GIVEN NAME) _____ (MIDDLE INITIAL OR NAME) Kudoh (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship Japan

Residence _____

Post Office Address _____

_____

Full name of 9th joint inventor, if any

_____ (GIVEN NAME) _____ (MIDDLE INITIAL OR NAME) _____ (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship _____

Residence _____

Post Office Address _____

2971064

Attorney's Docket No. U 012653-9 **PATENT**

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type:

(check one applicable item below)

- ☐ original.
- ☐ design.
- ☐ supplemental.

NOTE If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

- ☒ national stage of PCT.

NOTE If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P

- ☐ divisional.
- ☐ continuation.
- ☐ continuation-in-part (C-I-P).

INVENTORSHIP IDENTIFICATION

WARNING: *If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.*

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TITLE OF INVENTION

Novel gene defective in apeced and its use

SPECIFICATION IDENTIFICATION

the specification of which:

(complete (a), (b) or (c))

(a) ☐ is attached hereto.

(b) ☐ was filed on _____, as ☐ Serial No. 0 /_____ or ☐ Express Mail No., as Serial No. not yet known _____ and was amended on _____ *(if applicable)*.

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(c) ☒ was described and claimed in PCT International Application No. PCT/FI98/00749, filed on 23 Sept. 1998 and as amended under PCT Article 19 on _____ *(if any)*.

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56,

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☐ in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 CFR 1.98.

PRIORITY CLAIM (35 U.S.C. § 119(a)-(d))

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

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(d) ☐ no such applications have been filed.

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NOTE *Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.*

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119(a)-(d)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 USC 119
FI	973762	23 Sept. 1997	☒ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(34 U.S.C. § 119(e))

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER	FILING DATE
_____ /_____	_____
_____ /_____	_____
_____ /_____	_____

CLAIM FOR BENEFIT OF EARLIER US/PCT APPLICATION(S) UNDER 35 U.S.C. 120

☐ The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN PART (C-I-P) APPLICATION.

ALL FOREIGN APPLICATION(S), *IF ANY*, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

_____

_____

_____

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POWER OF ATTORNEY

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

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JOSEPH H. HANDELMAN, 26179
JOHN RICHARDS, 31053
JOHN J CHRYSTAL, 26360
RICHARD J STREIT, 25765
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RICHARD P. BERG, 28145
JULIAN H. COHEN, 20302
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2971064

Attorney's Docket No. U 012653-9 **PATENT**

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As a below named inventor, I hereby declare that:

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TITLE OF INVENTION

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SPECIFICATION IDENTIFICATION

the specification of which:

(complete (a), (b) or (c))

(a) ☐ is attached hereto.

(b) ☐ was filed on _____, as ☐ Serial No. 0 /_____ or ☐ Express Mail No., as Serial No. not yet known _____ and was amended on _____ *(if applicable)*.

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(also check the following items, if desired)

☐ and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and

☐ in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 CFR 1.98.

PRIORITY CLAIM (35 U.S.C. § 119(a)–(d))

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)–(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) ☐ no such applications have been filed.

(e) ☒ such applications have been filed as follows.

NOTE *Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.*

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119(a)-(d)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 USC 119
FI	973762	23 Sept. 1997	☒ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)

(34 U.S.C. § 119(e))

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER	FILING DATE
_____ /_____	_____
_____ /_____	_____
_____ /_____	_____

CLAIM FOR BENEFIT OF EARLIER US/PCT APPLICATION(S) UNDER 35 U.S.C. 120

☐ The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN PART (C-I-P) APPLICATION.

ALL FOREIGN APPLICATION(S), *IF ANY*, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

_____

_____

_____

NOTE. If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. § 120.

POWER OF ATTORNEY

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

PAUL B WEST, 18947
JOSEPH H. HANDELMAN, 26179
JOHN RICHARDS, 31053
JOHN J CHRYSTAL, 26360
RICHARD J STREIT, 25765
ALAN K ROBERTS, 17777
S. DELVALLE GOLDSMITH, 14383
PETER D. GALLOWAY, 27885
IAIN C. BAILLIE, 24090
THOMAS F. PETERSON, 24790
RICHARD P. BERG, 28145
JULIAN H. COHEN, 20302
WILLIAM R. EVANS, 25858
JANET I. CORD, 33778
CLIFFORD J. MASS, 30086

(check the following item, if applicable)

☐ Attached, as part of this declaration and power of attorney, is the authorization of the above-named attorney(s) to accept and follow instructions from my representative(s)

SEND CORRESPONDENCE TO

LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023

DIRECT TELEPHONE CALLS TO.
(Name and telephone number)

(212)708-1930

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

NOTE Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other documents.

Full name of sole or first inventor

Kai _____ Krohn
(GIVEN NAME) *(MIDDLE INITIAL OR NAME)* *FAMILY (OR LAST NAME)*

Inventor's signature _____

Date _____ **Country of Citizenship** Finland

Residence _____

Post Office Address _____

_____

Full name of second joint inventor, if any

Maarit _____ Heino
(GIVEN NAME) *(MIDDLE INITIAL OR NAME)* *FAMILY (OR LAST NAME)*

Inventor's signature _____

Date _____ **Country of Citizenship** Finland

Residence _____

Post Office Address _____

_____

Full name of third joint inventor, if any

Pärt _____ Peterson
(GIVEN NAME) *(MIDDLE INITIAL OR NAME)* *FAMILY (OR LAST NAME)*

Inventor's signature _____

Date _____ **Country of Citizenship** Estonia

Residence _____

Post Office Address _____

_____

(check proper box(es) for any of the following added page(s) that form a part of this declaration)

☒ **Signature** for fourth and subsequent joint inventors. *Number of pages added* 2

. . .

☐ **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* _____

. . .

☐ **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. *Number of pages added* _____

. . .

☐ Added page for **signature** by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 CFR 1.47)

. . .

☐ Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

☐ Number of pages added _____

. . .

☐ Authorization of attorney(s) to accept and follow instructions from representative.

. . .

(if no further pages form a part of this Declaration, then end this Declaration with this page and check the following item)

☐ This declaration ends with this page.

Attorney's Docket No. _____

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

Full name of fourth joint inventor, if any

Hamish (GIVEN NAME) _____ (MIDDLE INITIAL OR NAME) Scott (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship Australia

Residence _____

Post Office Address _____

_____

Full name of fifth joint inventor, if any

Stylianos (GIVEN NAME) _____ (MIDDLE INITIAL OR NAME) Antonarakis (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship US/Greece

Residence _____

Post Office Address _____

_____

Full name of sixth joint inventor, if any

Maria (GIVEN NAME) _____ (MIDDLE INITIAL OR NAME) Lalioti (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship _____

Residence _____

Post Office Address _____

Attorney's Docket No. _____

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

7-00

Full name of 7th joint inventor, if any

Nobuyoshi (GIVEN NAME) _____ (MIDDLE INITIAL OR NAME) Shimizu (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship Japan

Residence _____

Post Office Address _____

_____

8-00

Full name of 8th joint inventor, if any

Jun (GIVEN NAME) _____ (MIDDLE INITIAL OR NAME) Kudoh (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship Japan

Residence _____

Post Office Address _____

_____

Full name of 9th joint inventor, if any

_____ (GIVEN NAME) _____ (MIDDLE INITIAL OR NAME) _____ (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship _____

Residence _____

Post Office Address _____

5/9 FAX to
358-3-3131-7050

Attorney's Docket No. U 012653-9

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

Full name of 7th joint inventor, if any

Nobuyoshi		Shimizu
GIVEN NAME	MIDDLE INITIAL OR NAME	FAMILY (OR LAST NAME)

Inventor's signature Nobuyoshi Shimizu

Date May 5, 2000 Country of Citizenship Japan

Residence 4-1-W2103 Yukarigaoka, Sakura, Chiba 285

Post Office Address Japan

Full name of 8th joint inventor, if any

Jun		Kudoh
GIVEN NAME	MIDDLE INITIAL OR NAME	FAMILY (OR LAST NAME)

Inventor's signature Jun Kudoh

Date May 8, 2000 Country of Citizenship Japan

Residence E-14-8-303, Honkomagome, Bunkyo-ku, Tokyo 113-0021

Post Office Address Japan

Full name of 9th joint inventor, if any

GIVEN NAME	MIDDLE INITIAL OR NAME	FAMILY (OR LAST NAME)

Inventor's signature

Date Country of Citizenship

Residence

Post Office Address